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Phytochemical content and antioxidant activities of thirteen fruits of Assam, India

Sangeeta Saikia, Nikhil Kumar Mahnot, Charu Lata Mahanta *

Department of Food Engineering and Technology, Tezpur University, Assam, India

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ABSTRACT

Thirteen selected fruits were studied for their phytochemical content and antioxidant activity. The highest total phenolic content was observed in black jamun followed by litchi, *bogi jamun*, amla, hog plum, *pani jamun* and carambola. Amla showed the highest ferric reducing antioxidant potential value. DPPH radical scavenging activity of black jamun, litchi, amla, hogplum, and *poniol* was above 90%. The metal chelation capacity was highest in *poniol* (18.55%), carambola (15.95%) and *leteku* (11.54%). RP-HPLC study of the fruit extracts showed presence of ascorbic acid, phenolic acids and flavonoids. The composition and content of the phenolic acids varied depending on the fruit type.

1. Introduction

Epidemiological studies have established that the polyphenols, mainly phenolic acid and flavonoids present in fruits are effective in reducing the risk of chronic degenerative diseases (Wright et al., 2008). The polyphenols are the secondary plant metabolites and participate in metabolic functions including the assimilation of nutrients and formation of structural components and act as a defense against adverse environmental conditions (Simon-Graoa et al., 2014). Apart from imparting color, flavor and astringency (Robbins, 2003), the polyphenols present in fruits can scavenge the free radicals and destroy the oxidation pathways initiated by free radicals like lipid peroxidation and DNA damage in the human body. Such an action lowers the risk of cancer, cardiovascular diseases and problems associated with ageing (Leong & Shui, 2002). However, the effectiveness and activity against the free radicals depend on the polyphenol composition and content in the fruit. Different fruits contain different polyphenols and their radical scavenging property varies accordingly (Saura-Calixto & Goni, 2006). Earlier studies had suggested that the composition and content of polyphenols in fruits vary with cultivar, environmental conditions, location and agronomic factors (Naczk & Shahidi, 2006).

Varieties of fruits are available in Assam, situated in the North-eastern part of India. But their phytochemical content and antioxidant properties in the raw state have not been systematically studied. Various fruits are believed to have some therapeutic properties and are used in many traditional medicines. Study of their phytochemical properties is required to harness their goodness into the diet of the people. This study was carried out to estimate the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities of thirteen fresh fruit samples from Tezpur, Assam and also to determine ascorbic acid and major phenolic acids present in these fruits by RP-HPLC.

2. Materials and methods

All the chemicals used were of analytical grade and supplied by Merck, India, Himedia Laboratories and Sigma Chemicals, India.

2.1. Fruit samples

Bogi jamun (*Syzygium jambos* L.), amla (*Emblica officinalis* Gaertn), Indian olive (*Elaeocarpus serratus* L.), *leteku* (*Baccurea sapida* Muell. Arg), carambola (*Averrhoa carambola* L.), black jamun (*Syzygium cumuni* L. Skeels.), watermelon (*Citrullus lanatus* var *lanatus*), pineapple (*Ananas comosus L. Merr*), hog plum (*Spondias pinnata L. Kurz*), *pani jamun* or water apple (*Syzygium samarangense* (Blume) Merr. & Perry), Khasi mandarin orange (*Citrus reticulata* Blanco), *poniol* or coffee plum (*Flacourtia jangomas*

* Corresponding author. Fax: +91-3712-267005.
E-mail address: charu@tezu.ernet.in (C.L. Mahanta).

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(Lour.) Raeusch) and litchi (*Litchi chinensis* Sonn.) were procured from the local fruit market at Tezpur, Assam during the season.

2.2. Sample extraction

Each fresh fruit was homogenized and extracted in 80% acetone for 90 min at 20 °C in a ratio of 1:10 (sample:solvent) in a shaking incubator (Labtech) at 200 rpm and then centrifuged (Hettich, Germany) at 970*g*. The supernatant was collected and stored at −20 °C until further analysis.

2.3. Determination of total phenolic content

Total phenolic content in the sample extracts was assessed following the Folin–Ciocalteau assay (Slinkard & Singleton, 1977) with slight modification. For the analysis, 20 μl each of sample extract, gallic acid standard or blank were taken in separate test tubes and to each 1.58 ml of distilled water and 100 μl of Folin–Ciocalteau reagent were added, mixed well and within 8 min, 300 μl of sodium carbonate was added. The samples were vortexed immediately and the tubes were incubated in the dark for 30 min at 40 °C. The absorbance was then measured at 765 nm in a UV–vis spectrophotometer (Cecil, Aquarius 7400). The results were expressed in mg GAE/ 100 g.

2.4. Determination of total flavonoid content

The flavonoid content was determined by aluminum trichloride method (Chang, Yang, Wen, & Chern, 2002). Briefly, 0.5 ml of the sample extract was mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum trichloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of deionised water. After incubation at room temperature for 40 min, the absorbance of the reaction mixture was measured at 415 nm against deionised water taken as blank in a UV–vis spectrophotometer (Cecil, Aquarius 7400). Results were expressed as quercetin equivalent (mg QE/100 g) of sample.

2.5. Determination of antioxidant property of sample extracts

2.5.1. Ferric reducing antioxidant property (FRAP)

FRAP activity of the samples was measured by the method of Benzie and Strain, (1996). Briefly, a 40 μl aliquot of properly diluted sample extract was mixed with 3 ml of FRAP solution. The reaction mixture was incubated at 37 °C for 4 min and the absorbance was determined at 593 nm in a UV–vis spectrophotometer (Cecil, Aquarius 7400) against a blank that was prepared using distilled water. FRAP solution was pre warmed at 37 °C and prepared freshly by mixing 2.5 ml of a 10 mM 2,4,6-TPTZ [2,4,6-tri(2-pyridyl)-1,3,5-triazine] solution in 40 mM hydrochloric acid with 2.5 ml of 20 mM ferric chloride and 25 ml of 0.3 M acetate buffer (pH 3.6). A calibration curve was prepared, using an aqueous solution of ferrous sulfate (1–10 mM). FRAP values were expressed as μM of ferrous equivalent Fe (II) per 100 g of sample.

2.5.2. DPPH radical scavenging activity

Radical scavenging activity of the sample extracts was measured by determining the inhibition rate of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical (Brand-Williams, Cuvelier, & Berset, 1995). Precisely, 100 μl of extracts was added to 1.4 ml DPPH radical methanolic solution (10^{-4} M). The absorbance at 517 nm was measured at 30 min against blank (100 μl methanol in 1.4 ml of DPPH radical solution) using a UV–vis Spectrophotometer (Cecil Aquarius 7400). The results were expressed in terms of radical scavenging activity.

Radical scavenging activity (%) = [(Ao − As)/Ao] × 100

where Ao is absorbance of control blank, and As is absorbance of sample extract.

2.5.3. Metal chelation capacity

Metal chelating capacity was determined based on the method of Dinis, Madeira, and Almeida (1994). For the estimation, 1.0 ml of 0.125 mM ferrous sulfate, and 1.0 ml of 0.3125 mM ferrozine were mixed with 0.2 ml sample extract. The mixture was allowed to equilibrate for 10 min at room temperature and the absorbance at 562 nm in a UV–vis spectrophotometer (Cecil, Aquarius 7400) was recorded. The control contained all the reaction reagents except the extract. Decreased absorbance of the reaction mixture indicated increased activity.

Chelation activity [%] = [(Ao − As)/Ao] × 100

where Ao is absorbance of control blank, and As is absorbance of sample extract.

2.6. RP-HPLC study of the polyphenols

The sample extract was prepared by extracting the fruits in 80% acetone. The extract was evaporated under vacuum, redissolved in 1 ml of HPLC grade methanol and filtered through a 0.22 μm nylon filter (Himedia, India). RP-HPLC (Waters system) gradient elution method was used to identify the major phenolic acid composition of the studied samples. Symmetry 300^{TM} C_{18} (5 μm, 4.6 mm × 250 mm) column with a binary pump (Waters, 1525) and a UV–vis detector (Waters, 2489) were used. Mobile phases used were acidified ultrapure water (0.1% acetic acid, pH 3.2, mobile phase A) and methanol (mobile phase B). The gradient method of Saikia, Mahnot, and Mahanta (2015) was followed with 80% A (0–8 min), 65% A (9–12 min), 45% A (13–16 min), 30% A (17–20 min), 20% A (21–30 min), 10% of A (31–34 min) and then washing of the column with 65% A (35–39 min) and lastly with 80% A (40–45 min). Sample volume of 20 μl was used. The flow rate was maintained at 0.8 ml/min and wavelengths used for UV–vis detector were 254 nm and 325 nm. The standards used for comparison and identification were gallic acid, ascorbic acid, catechin, chlorogenic acid, caffeic acid, syringic acid, ferulic acid, coumaric acid, rutin hydrate, kaempferol and quercetin.

2.7. Statistical analysis

All experiments were carried out at least in triplicates and reported as mean ± standard deviation of mean (S.E.M.) using SPSS version 11.5. The data were statistically analyzed by Duncan's multiple range tests at $p \leq 0.05$ significant levels.

3. Results and discussion

3.1. Phytochemical and antioxidant properties of fruit samples

The highest TPC was observed in black jamun followed by litchi, *bogi jamun*, amla, hogplum, *pani jamun*, carambola, *poniol* and *leteku* (Table 1). The lowest TPC value was observed in watermelon. The highest flavonoid content was found in amla followed by hogplum, black jamun, *leteku*, olive and carambola.

The highest FRAP value was observed in amla followed by black jamun, hogplum, carambola, *poniol*, *bogi jamun* and *leteku* (Table 2). Similarly, black jamun, litchi, amla, hogplum, *poniol* showed DPPH activity above 90% while pineapple gave the lowest activity of 22.30%. The MCC value was highest in *poniol* (18.55%), carambola (15.95%) and *leteku* (11.54%).

The studied fruit samples were divided according to their phenolic content (Rufino et al., 2010) based on fresh matter into three categories: low (< 100 mg GAE/100 g), medium (100–500 mg GAE/100 g) and high (> 500 mg GAE/100 g). Olive, Khasi mandarin and