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Antioxidant properties of selected fruit cultivars grown in Sri Lanka

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2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (PubChem CID: 77258)
Folin-Ciocalteu's reagent (PubChem CID: 516996)
Aluminum trichloride (PubChem CID: 24012)
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Gallic acid (PubChem CID: 370)
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ABSTRACT

Extracts of twenty locally available Sri Lankan fruits were analysed for 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, ferrous reducing antioxidant power (FRAP), total phenolic content (TPC), total flavonoid content (TFC) and vitamin C content. The results showed that gooseberry (*Phyllanthus emblica* 'local') exhibited the highest DPPH scavenging activity (111.25 mg ascorbic acid equivalent antioxidant capacity (AEAC)/g), FRAP (1022.05 μmol $FeSO_4$/g), TPC (915.7 mg gallic acid equivalents (GAE)/100 g), TFC (873.2 mg catechin equivalents (CE)/100 g) and vitamin C (136.8 mg ascorbic acid equivalents (AAE)/100 g), respectively. Sugar apple (*Annona squamosa* 'local') and star fruit (*Averrhoa carambola* 'Honey Sweet') obtained the second and third highest antioxidant activities in terms of rankings of FRAP, DPPH activities, TPC, TFC and vitamin C content. Strong correlation between vitamin C, TPC and TFC with FRAP and DPPH showed their contribution to antioxidant capacity. Among the selected fruits, underutilized fruit cultivar gooseberry showed the highest overall antioxidant potential.

1. Introduction

Fruits are rich in antioxidants which can prevent or delay oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species and non-radicals. Antioxidants scavenge radicals by inhibiting initiation and breaking chain propagation or suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxides and quenching superoxide and singlet oxygen (Shi, Noguchi, & Niki, 2001). Therefore, antioxidants help in lowering the incidence of degenerative diseases such as cancer (Benetou et al., 2008), arthritis (Cerhan, Saag, Merlino, Mikuls, & Criswell, 2003), atherosclerosis (Dauchet, Amouyel, & Dallongeville, 2009; Ruel & Couillard, 2007), inflammation (González-Gallego, García-Mediavilla, Sánchez-Campos, & Tuñón, 2010), brain dysfunction (Lau, Shukitt-Hale, & Joseph, 2007) and acceleration of the ageing process (Paredes-López, Cervantes-Ceja, Vigna-Pérez, & Hernández-Pérez, 2010). The most abundant antioxidants in fruits are polyphenols and vitamin C. The fruit polyphenols are the most important group of natural antioxidants because of their diversity

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and extensive distribution, and they possess the ability to scavenge both active oxygen species and electrophiles.

Sri Lanka is a tropical country with a large diversity of fruits. Fruits from the tropical climates are known to be associated with many medicinal properties. Besides the commonly consumed fruits, some underutilized fruits are known in traditional foods, especially in rural communities. Sri Lankan native fruits and vegetables are still being used in traditional indigenous medicine. These are rarely eaten, unknown, and unfamiliar and have not received much attention as antioxidant sources compared to commercial fruits. This could be due to their lack of popularity among local communities and lack of information on nutritional compositions and physical qualities. Therefore, determination of antioxidant properties of Sri Lankan local fruits is important.

Since the research on antioxidant properties of underutilized fruits in Sri Lanka is scarce (Gunawardena & Silva, 2006), it is crucial to identify the antioxidant properties of Sri Lankan indigenous traditional fruits and this could potentially be used to address the rapid rate of increase in non-communicable diseases in Sri Lanka. Dissemination of the knowledge on health promoting properties of those fruits will possibly promote the cultivation and commercialization of them to increase their consumption at local and national level.

Evaluation of antioxidant capacity of plant foods cannot be done accurately by any single method due to the complex nature of phytochemicals and involvement of multiple reaction characteristics and mechanisms. Therefore, a combination of assays will provide precise information of the antioxidant properties of fruits. The objective of the present study was to determine the antioxidant properties of selected locally available common and underutilized fruit cultivars in Sri Lanka.

2. Materials and methods

2.1. Fruits

Twenty locally available Sri Lankan fruit cultivars: bilimbi (*Averrhoa bilimbi* 'local'), Ceylon date palm (*Phoenix pusilla* 'local'), Ceylon olive (*Elaeocarpus serratus* 'local'), sugar apple (*Annona squamosa* 'local'), mandarin (*Citrus reticulate* 'local'), pummelo (*Citrus maxima* 'local'), miracle berry (*Synsepalum dulcificum* 'local'), bullock's heart (*Annona reticulata* 'local'), soursop (*Annona muricata* 'local'), Jamaica plum (*Spondias dulcis* 'local'), gooseberry (*Phyllanthus emblica* 'local'), governor's plum (*Flacourtia indica* 'local'), mango (*Mangifera indica* 'Villard'), guava (*Psidium guajava* 'Horana white'), star fruit (*Averrhoa carambola* 'Honey Sweet'), rambutan (*Nephelium lappaceum* 'Malayan red'), pomegranate (*Punica granatum* 'Daya'), banana (*Musa paradisiacal* 'Kolikuttu'), rose apple (*Syzygium jambos* 'Malaysian') and grapes (*Vitis vinifera* 'Israel blue') were collected from Fruits Research Institute of Department of Agriculture, Sri Lanka and home-gardens in Northern and North-Western Provinces of Sri Lanka. All the fruits were harvested freshly in the fully matured and ripened stage, transported to the laboratory in ice boxes, kept in a refrigerator and extracted within two days after acquisition. Extractions were stored at −80 °C until analysis.

2.2. Chemical reagents

Methanol, metaphosphoric acid, ascorbic acid, 2,2-diphenyl-1-picrylhydrazil (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), Folin-Ciocalteu's reagent, aluminium trichloride, 2,6-dichloroindophenol, gallic acid (97.5–102.5%) and catechin (⩾98%) were purchased from Sigma-Aldrich. All chemicals and reagents used in the study were of analytical grade.

2.3. Sample preparation

2.3.1. Extraction for Vitamin C analysis

The procedure developed by (Masamba & Nguyen, 2008) was employed after slight modifications. Briefly, edible portions (2.5 g) of each of the fruit samples were accurately weighed and ground using mortar and pestle with an addition of 5 ml of freshly prepared metaphosphoric acid-acetic acid. The mixture was further ground and strained through muslin and the extract was made up to 25 ml with the metaphosphoric acid-acetic acid mixture. This extraction procedure was repeated two more times. The extracts were recovered and stored at −80 °C until analysis. These extracts were used for the determination of vitamin C content. During all stages, extracts were protected from light by covering them with Aluminium foil.

2.3.2. Extraction using methanol for total phenolic content, total flavonoid content, DPPH and FRAP analysis

Methanolic extraction was performed according to the method elaborated by Chandrasekara and Shahidi (2010) with slight modifications. Briefly, approximately 1 g of homogenised ground fruit sample was weighed and mixed with 10 ml of 80% (v/v) of methanol and placed on a shaking water bath at 60 °C, 50 rpm for 25 min. Then it was centrifuged for 10 min at 4000*g*. Supernatant was collected and extraction was repeated two more times. The supernatants were recovered and stored at −80 °C until analysis after volume up to 50 ml by using 80% of methanol. These extracts were used for the determination of total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP) value and (2,2-diphenyl-1-picryl-hydrazyl-hydrate) DPPH scavenging activity of fruits. During all stages, extracts were protected from light by covering them with Aluminium foil.

2.4. Sample analysis

2.4.1. Determination of vitamin C content

Vitamin C content was determined by AOAC official titrimetric Method 967.21 for ascorbic acid in vitamin preparations and juices (AOAC, 1995). Briefly, 5 ml of metaphosphoric acid acetic acid solution was pipetted to an Erlenmeyer flask, which contains 2 ml of extract. The mixture was titrated with 2,6-dichloroindophenol dye solution until a light rose pink colour persisted for 5 s. The amount of dye used in the titration was determined and used in the calculation of vitamin C content. The vitamin C content was expressed in mg of ascorbic acid equivalents (AAE) per g of fresh fruit sample. Blanks were prepared by adding 5 ml of metaphosphoric acid acetic acid and 2 ml of deionized water.

2.4.2. Determination of TPC

The TPC of each extract was determined using a modified method of (Singleton & Rossi, 1965) based on the reduction of a phosphowolframate phosphomolibdate complex by phenolics to blue reaction products (Lim, Lim, & Tee, 2007). Briefly, 300 µl of standard or sample was pipetted out into the centrifuge tube and 1.5 ml of Folin-Ciocalteu was added, followed by 1.2 ml of Na_2CO_3 (7.5% w/v). Then the solution was vortexed for few seconds and immediately incubated for 35 min at room temperature in the dark. After incubation the mixture was centrifuged at 4000 g for 5 min and absorbance was measured at 765 nm wave length using a spectrophotometer (Spectro UV–vis Auto UV-2602, USA). Methanol (80%) was used as the blank. Total phenol content was estimated from a standard curve of gallic acid and results expressed in gallic acid equivalents (GAE) (mg per 100 g fresh fruit).