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Nutritional Composition and Antioxidant Activity of Selected Underutilized Fruits Grown in Sri Lanka

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Abstract: Sri Lanka has been identified as one of the world's biodiversity hotspots with a rich array of fruits; however, most of them remain underutilized. The present study was carried out to investigate the nutritional composition and to assess the bioactivity of six underutilized fruits collected from three provinces of Sri Lanka. The selected fruit species were Màdan (Syzygium cumini), Maha Karamba (Carissa carandas), Himbutu (Salacia chinensis), Ugurassa (Flacourtia indica), Barbados cherry (Malpighia emarginata), and Ceylon gooseberry (Dovyalis hebecarpa). The proximate composition, total dietary fiber content, mineral content, total phenolic content, and vanillin contents were determined using standard methods. The antioxidant activity of methanolic extracts of fruits was assessed using FRAP and DPPH assays. Uguressa extract exhibited the highest dietary fiber content (12.25 ± 0.29 of fresh fruit weight) while the lowest was observed in Barbados cherry (6.01 ± 1.10 g/100 g). The total phenolic content (TPC) of fruits ranged from 6.8 ± 0.4 to 10.3 ± 0.3 milligram gallic acid equivalents/g fruit. Barbados cherry showed the highest antioxidant activity (AOA) as measured by FRAP (0.022 ± 0.003 mM Fe^{2+}/g fruit) and the highest vanillin content (2.4 mg/kg). The highest potassium (434.60 ± 0.36 mg/kg), phosphorous (16.69 ± 0.46), and calcium contents (23.43 ± 0.45) were observed in Uguressa. Màdan had the highest content of magnesium (13.25 ± 0.38 mg/kg), sodium (5.28 ± 0.30), iron (0.65 ± 0.12 mg/kg), and aluminum (1.15 ± 0.16 mg/kg). The highest manganese content (0.98 ± 0.18 mg/kg) was observed in Himbutu while the highest copper content was found in Uguressa (0.11 ± 0.04 mg/kg) and Maha Karamba (0.11 ± 0.03 mg/kg). The study reveals that six underutilized fruits tested possess high nutritional value and are rich in antioxidant activity.

Keywords: antioxidant activity; dietary fiber; mineral content; underutilized fruits; vanillin

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1. Introduction

Fruits and vegetables contain dietary fiber and a wide array of antioxidants. Epidemiological studies have shown that a high intake of fresh fruits and vegetables is associated with a lower risk of mortality from cancer and coronary heart diseases [1]. Dietary fiber intake also significantly reduces the risk of developing stroke, hypertension, diabetes, obesity, and certain gastrointestinal diseases [2–4]. Phytochemicals exhibiting antioxidant properties

effectively scavenge free radicals and thereby mitigate oxidative damage to biomolecules, leading to reduced age-related disease conditions, such as cancer and atherosclerosis [1,5].

Sri Lanka has been identified as a biodiversity hotspot [6,7]. A large number of wild fruit species distributed throughout Sri Lanka have contributed to the rich biodiversity of the country. Most of these fruits remain underutilized and are sold exclusively at local village markets during the fruiting season. These fruits are rich in nutrients; however, they are neither cultivated on a large scale, nor are they commonly available in regular markets. These fruit species have been used for centuries in the country for food, fodder, fiber, and medicinal uses; nevertheless, their use has been reduced over the past few decades due to urbanization, land clearance, and the commercialization of a few selected fruits [7]. Some of the underutilized fruits grown in Sri Lanka include Lovi (*Flacourtia inermis*), Himbutu (*Salacia chinensis*), Mora (*Euphoria longan*), Uguressa (*Flacourtia indica*), Pomelo (*Citrus maxima*), Wali anoda (*Annona reticulata* L.), Màdan (*Syzygium cumini*), Masan (*Ziziphus mauritiana* var. *mauritiana*), Kirala (*Snnaeratia alba*), Weera (*Drypetes sepiaria*), Palu (*Manilkara hexandra*) and Kon (*Schleichera oleosa*) [8]. Many of the underutilized fruits are not only rich sources of fiber, vitamins, and minerals but also contain bioactive plant compounds such as antioxidants, anti-cancer, and antimicrobial constituents [9–11]. Himbutu and Ceylon Gooseberry are under the category of berries among the tested fruits. According to the studies conducted on wild berries, these are found to be rich in phenolic compounds such as phenolic acids, tannins, stilbenes, anthocyanins, and flavonoids. Berries have been the focus of considerable research as rich sources of anthocyanin [12–14]. Despite the rich-ness of the underutilized fruits, to date there has been little information available on their nutritional value and bioactive properties.

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is known to have anticarcinogenic, phenolic, and antioxidant properties and it is one of the strong bioactive plant compounds present in many fruits [15–19] in their free form or as glucosides in mangos [20], blueberries [21], strawberries [22], and lychees [23]. No data are available on the vanillin content of underutilized fruits in Sri Lanka. Hence, this study was carried out to determine the proximate composition and assess the antioxidant activity of six selected underutilized fruit species grown in Sri Lanka, namely, Màdan (*Syzygium cumini*), Maha karamba (*Carissa carandas*), Himbutu (*Salacia chinensis*), Ugurassa (*Flacourtia indica*), Barbados cherry (*Malpighia emarginata*), and Ceylon gooseberry (*Dovyalis hebecarpa*).

2. Materials and Methods

2.1. Sample Collection

Approximately 100 g of healthy, ripened, undamaged fruits (Figure 1) were collected from different locations after the plants were authenticated by the Plant Genetics Resources Center, Gannorwa. Màdan was collected from home gardens in North Central province (Anuradhapura); Ugurassa, Mahakaramba, Ceylon gooseberry, and Barbados cherry from Central province (Katugastota, Uda Peradeniya and the Horticultural Research and Development Institute, Gannoruwa, respectively); and Himbutu from Sabaragamuwa province (Belihuloya), Sri Lanka. As maturity indices for these fruits are not available, fruits ready for consumption were selected for the analysis.

2.2. Chemicals and Reagents

Ethanol of analytical grade (AR) was purchased from AnalaR NORMAPUR (VWR BDH, Bridgeport, NJ 08014, Philadelphia, PA, USA). Petroleum ether, sulfuric acid, potassium sulfate, copper sulfate, sodium hydroxide, hydrochloric acid, monosodium phosphate (NaH_2PO_4), disodium phosphate (Na_2HPO_4), acetone, Folin–Ciocalteu reagent, sodium carbonate, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent, sodium acetate, acetic acid, 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), Ferric chloride hexahydrate ($FeCl_3.6H_2O$), ferric sulfate, and nitric acid of AR grade; methanol, phosphoric acid, and acetonitrile of HPLC grade; vanillin (4-hydroxy-3-methoxybenzaldehyde) (V1104) of reagent plus grade; and heat-stable α-amylase (A3306), protease (P3910), and amyloglucosidase (A9913) for the

Total Dietary Fiber Assay (TDF-100A) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All chemicals used were of the highest purity available.

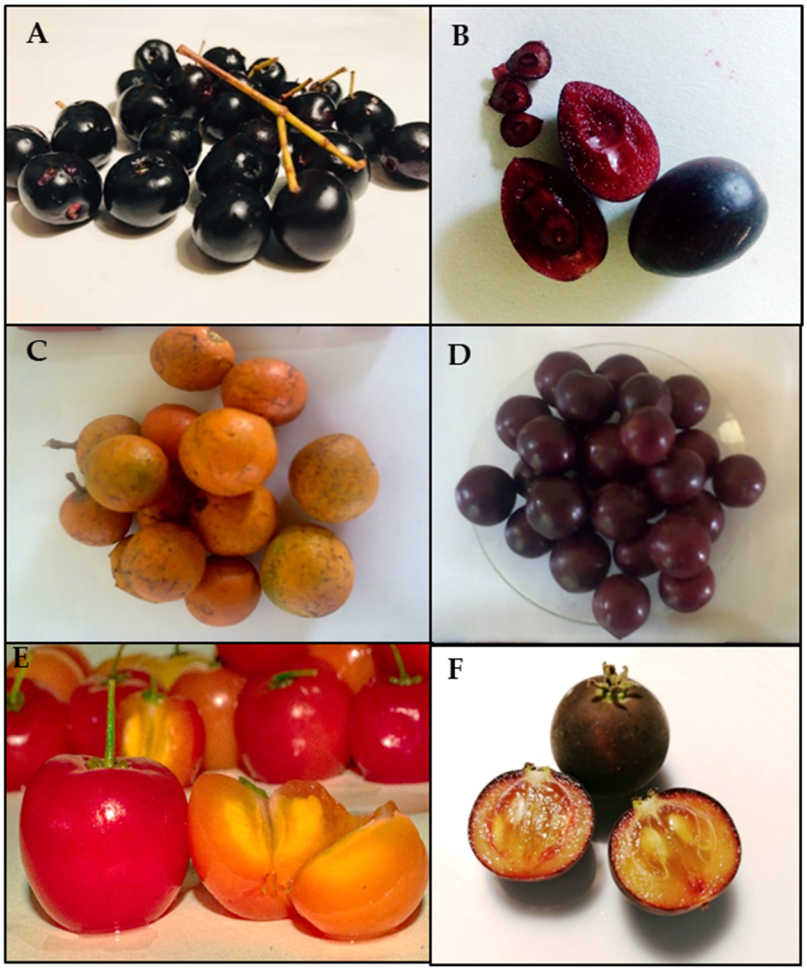

Figure 1. Fruits of each species used in the study: (**A**) Màdan (*Syzygium cumini*), (**B**) Maha karamba (*Carissa carandas*), (**C**) Himbutu (*Salacia chinensis*), (**D**) Ugurassa (*Flacourtia indica*), (**E**) Barbados cherry (*Malpighia emarginata*), (**F**) Ceylon gooseberry (*Dovyalis hebecarpa*).

2.3. Sample Preparation

Healthy, ripened fruits were washed and the non-edible parts such as skin and seeds were removed. The edible parts were homogenized in a blender for 3 min to obtain 100 g of fruit pulp. A portion of pulp was oven-dried using Memmert-UFE 600, Burladingen/Deutschland, Germany (AOAC 930.15) [24], vacuum packed, and used for proximate analysis, as well as for the determination of dietary fiber content. The rest of the pulp was vacuum-packed in plastic bags and stored at −18 °C until further analysis for antioxidants, which was completed within 2 months. All analyses were carried out in triplicate.

2.4. Determination of Proximate Composition

The moisture content was determined using the oven (Memmert-UFE 600, Burladingen/Deutschland, Germany) dry method (AOAC 930.15) [24]. The ash content was determined using AOAC 942.05 [25] in a muffle furnace (Thomas Scientific, Swedesboro, NJ, USA). The fat content was determined gravimetrically via the Soxtec method (AOAC 2003.05) [26] using Soxtec equipment (SoxtecTM 2055, Foss, Höganäs-Helsingborg, Sweden). The protein content was determined via the Kjeldahl method (AOAC 995.04) [27] with slight modifications, using a Kjeldahl apparatus (DK 6, F30100182 Series and UDK 129-F30200120 Series Velp Scientifica, Usmate (MB), Italy).

2.5. Determination of Total Dietary Fiber (TDF) Content

The total dietary fiber content was determined via the enzymatic gravimetric method (AOAC 985.29) [28] using a dietary fiber analyzer (Enzymatic digester-GDE, SA30400209 series, and filtration unit- CSF6, F30420210 series Velp Scientifica, Usmate (MB), Italy). One gram of oven dried fruit sample was weighed, suspended in phosphate buffer (0.08 M, pH 6.0), and digested sequentially with heat-stable α-amylase at 100 °C for 30 min. Afterwards, it was digested with protease at pH 7.3–7.7 and 60 °C for 30 min, and then with amyloglucosidase at pH 4–4.6 and 60 °C for 30 min to remove protein and starch. The digests were mixed with ethanol (95%, 280 mL) at 60 °C. After 60 min, the residue was filtered through fritted glass crucibles and was rinsed with 78% and 95% ethanol, respectively. This was then rinsed with acetone. The residue was dried overnight at 105 °C in a forced-air oven and weighed to the nearest 0.1 mg. Out of the quadruplicate residues, two were ashed in a muffle furnace (AOAC 942.05) [25] at 525 °C. The other two samples were used to determine the protein content of the residue (AOAC 955.04) [29] as described above.

2.6. Preparation of Plant Extracts for Antioxidant and Total Phenolic Content

Phenolic compounds were extracted with aqueous methanol according to the method reported by Canuto et al. [30], with slight modifications. Exactly 4 g of the homogenized sample was mixed with 100 mL of 70% (*v*/*v*) methanol in a 250 mL conical flask by shaking for 2 h at 200 °C in a shaking water bath, followed by centrifuging (Hitachi, Himac CT 4Dd, Ibaraki 312-8502, Japan) at 3500 rpm for 15 min at room temperature. The resulting supernatant was collected, and extraction was repeated two more times. The supernatants were combined and stored at −20 °C until analysis.

2.7. Determination of Total Phenolic Content (TPC)

The total phenolic content of the fruit extracts was determined colorimetrically with slight modifications [31]. Fruit extracts (15 µL) were mixed with 70 µL of Folin–Ciocalteu reagent for 3 min and, subsequently, 215 µL of sodium carbonate (0.7 M) was added and mixed well. The absorbance of the resulting mixture was read at 760 nm using a UV visible spectrophotometer (AE270-2U, Thermo Scientific Multiskan Go, Ratastie 2, FI-01621, Finland) after leaving it for 30 min at room temperature (25 °C). The results were expressed as mg gallic acid equivalents (GAE) per gram of fresh fruit using a gallic acid (50–500 mg/L) standard curve.

2.8. Determination of Antioxidant Activity

2.8.1. Determination of DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

The DPPH radical scavenging assay was performed according to the method described by Deng et al. [32] with slight modifications. Methanolic DPPH solution (250 µL, 0.1 mM) was added to five different concentrations of fruit extracts (50 µL) ranging between 0.1 and 1.0 mg/mL and allowed to stand for 30 min in the dark at room temperature. The absorbance was read at 517 nm using a UV visible spectrophotometer (AE-270-2U, Thermo Scientific Multiskan Go, Ratastie 2, FI-01621, Finland). The sample concentration providing 50% inhibition (IC_{50}) was calculated using the standard calibration curve.

2.8.2. Determination of Ferric Reducing Antioxidant Power (FRAP)

The ability to reduce ferric ions was measured using the method described by Benzie and Strain [33] with some modifications. The FRAP reagent was prepared by mixing 300 mM sodium acetate buffer (pH 3.6), 10.0 mM tripyridyl triazine (TPTZ), and 20.0 mM $FeCl_3 \cdot 6H_2O$ solution at a ratio of 10:1:1. The prepared FRAP reagent (150 µL) was added to 100 µL of fruit extract and the reaction mixture was incubated at 37 °C for 30 min. The absorbance was read at 593 nm using a UV visible spectrophotometer (AE-270-2U, Thermo Scientific Multiskan Go, Ratastie 2, FI-01621, Finland). Fresh working solutions from a standard solution of $FeSO_4$ were used for the standard curve (0.2–1.0 M). The results were expressed as mM Fe^{2+} equivalents per gram of sample.

2.9. Quantification of Vanillin

Methanolic extracts of fruits were filtered through a 0.45 µm pore-size syringe filter and the solvent of 10 mL fruit extract was completely evaporated using nitrogen gas. The concentrate was dissolved in methanol (70%, 1 mL) and used for the analysis. Vanillin was quantified using the method described by Waliszewski et al. [34]. A Shimadzu Nexera-XR HPLC consisting of a LC-20ADxr pump, SPD-20AV UV detector, a CBM-20A/CBM-20Alite system controller, Lab Solutions software, and an auto sample injector was used. Vanillin (99.9% purity) stock solution (1 mg/mL) was prepared and stored in an amber-colored bottle at 4 °C. Water: methanol at 40:60 was used for isocratic elution to quantify vanillin in fruit extracts since this mixture showed the best resolving ability. An Ultra C18 column 250 × 4.6 mm, 5 µm (Restek Corp, Bellefonte, PA, USA) was used to separate vanillin at a column oven temperature of 37 °C, the wavelength of 231 nm, and a flow rate of 0.4 mL/min [35].

2.10. Determination of Mineral Content by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

The determination of mineral content was carried out using the method described by Zarcinas et al. [36] with slight modifications. Accurately 1.0 g of fruit sample was weighed into 50 mL Taylor tubes. Ten milliliters of conc. nitric acid 70% *v/v*) was added to each test tube and was heated for 45 min at 90 °C. The temperature was increased to 140 °C and digestion was continued until about 1 mL of acid remained. The digest was kept for cooling and was diluted to 20 mL with 1% *v/v* nitric acid. ICP-MS (Shimadzu-2030, Nakagyo-ku, Kyoto 604-8511, Japan) was used for the analysis of minerals. Stock solutions of 1000 mg/kg of the relevant standard (Na, P, K, Ca, Mg, Fe, Al, Mn, and Cu) were used for the preparation of calibration curves. Necessary dilutions were completed to obtain absorbance values and the mineral concentration was given in mg/kg. The method was validated by spiking NIST Standard Reference Materials of known concentrations.

2.11. Statistical Analysis

All assays were carried out in triplicate and the results were expressed as the mean ±SD. The mean values of each parameter were compared using Tukey's HSD. Significance was tested below the 0.05 level. The statistical analysis was carried out using Microsoft (MS) Excel 2016 for Windows and International Business Machines Corporation (IBM, Armonk, NY, USA) SPSS Version 23 for Windows.

3. Results

3.1. Proximate Composition

The moisture, ash, protein, and fat content of six underutilized fruits per 100 g of fresh fruit weight (FW) are presented in Table 1. The moisture content of fruits tested varied from 78.61 ± 1.17 to 88.01 ± 0.48 g per 100 g of FW. Barbados Cherry showed significantly ($p < 0.05$) higher moisture content than Màdan, Uguressa, Maha Karamba, and Himbutu, whereas compared to Uguressa, Maha Karamba, and Himbutu, Ceylon Gooseberry had a significantly ($p < 0.05$) high moisture content.

Table 1. Proximate composition (% of fresh) of six underutilized fruits.

Fruit	Moisture	Ash	Protein	Fat
Màdan	82.65 ± 1.67 ^{a,b}	4.67 ± 0.44 ^{b}	0.18 ± 0.01 ^{a}	1.62 ± 0.22 ^{b}
Uguressa	78.61 ± 1.17 ^{a}	3.17 ± 0.43 ^{a}	0.53 ± 0.03 ^{b}	0.5 ± 0.08 ^{a,b}
Maha karamba	79.37 ± 0.28 ^{a}	5.74 ± 0.04 ^{c}	0.92 ± 0.03 ^{c}	4.58 ± 0.06 ^{c}
Himbutu	80.83 ± 1.06 ^{a}	3.34 ± 0.47 ^{a}	1.15 ± 0.05 ^{d}	0.42 ± 0.13 ^{a,b}
Barbados cherry	88.01 ± 0.48 ^{c}	3.01 ± 0.25 ^{a}	1.24 ± 0.13 ^{d}	0.18 ± 0.02 ^{a}
Ceylon gooseberry	85.71 ± 0.48 ^{b,c}	5.66 ± 0.29 ^{c}	1.05 ± 0.09 ^{c,d}	0.22 ± 0.02 ^{a,b}

Values in Table 1 represent the mean ± SD of triplicate analysis; $n = 3$. Means within each column bearing different superscripts are significantly ($p < 0.05$) different.

According to Table 1, the ash content ranged from 3.01 ± 0.25 to 5.74 ± 0.04 g per 100 g of FW. Maha Karamba and Ceylon Gooseberry contained significantly higher ash content compared with other fruits analyzed ($p < 0.05$). Protein content varied from 0.18 ± 0.01 to 1.24 ± 0.13 g per 100 g of FW. Barbados cherry had a significantly higher protein content than Màdan, with a mean difference of 1.055, whereas Himbutu had a significantly high protein content compared with Màdan, Uguressa, and Maha Karamba. The fat content of the tested fruits varied from 0.18 ± 0.02 to 4.58 ± 0.06 g per 100 g of FW. All other fruits analyzed (Màdan, Uguressa, Himbutu, Barbodos Cherry, and Ceylon gooseberry) possessed a significantly lower fat content than Maha Karamba (4.58 ± 0.06 g) with a mean difference ranging from 2.96 to 4.40 (significant value is considered at $p < 0.05$).

3.2. Total Dietary Fiber (TDF) Content

Dietary fiber content varied from 5.37 ± 2.53 g (Ceylon gooseberry) to 12.25 ± 0.29 g (Uguressa) per 100 g of FW, as shown in Table 2. Uguressa had a significantly higher dietary fiber content than all other fruits analyzed, with the mean difference ranging from 3.99 to 6.88 ($p < 0.05$).

Table 2. Total dietary fiber content (% of fresh).

Fruit	Dietary Fiber Content
Màdan (*Syzygium cumini*)	6.23 ± 0.21 ^{a,b}
Ugurassa (*Flacourtia indica*)	12.25 ± 0.29 ^{c}
Maha karamba (*Carissa carandas*)	8.26 ± 0.87 ^{b}
Himbutu (*Salacia chinensis*)	6.07 ± 0.63 ^{a,b}
Barbados cherry (*Malpighia emarginata*)	6.01 ± 1.10 ^{a}
Ceylon gooseberry (*Dovyalis hebecarpa*)	5.37 ± 2.53 ^{a}

Values in Table 2 represent the mean ± SD of triplicate analysis; $n = 3$. Means bearing different superscripts are significantly ($p < 0.05$) different.

3.3. Total Phenolic Content (TPC) and Antioxidant Activity (FRAP and DPPH)

Total phenolic content and antioxidant activity are shown in Table 3.

Table 3. Total phenolic content and antioxidant activity.

Fruits	TPC mg GAE/g Fruit	FRAP mM Fe $^{2+}$/g Fruit	DPPH IC_{50} (mg/mL)
Màdan	8.901 ± 0.81 ^{a}	0.020 ± 0.003 ^{a}	0.067 ± 0.001 ^{b}
Uguressa	8.137 ± 0.89 ^{a}	0.015 ± 0.003 ^{a}	0.089 ± 0.001 ^{c}
Maha karamba	7.153 ± 0.21 ^{a}	0.020 ± 0.002 ^{a}	0.072 ± 0.001 ^{b}
Himbutu	9.148 ± 0.10 ^{a}	0.018 ± 0.003 ^{a}	0.043 ± 0.004 ^{a}
Barbados cherry	10.295 ± 0.29 ^{a}	0.022 ± 0.003 ^{a}	0.040 ± 0.002 ^{a}
Ceylon gooseberry	6.770 ± 0.38 ^{a}	0.020 ± 0.004 ^{a}	0.070 ± 0.001 ^{b}

Values represent the mean ± SD of triplicate analysis; $n = 3$. Means bearing different superscripts are significantly ($p < 0.05$) different.

3.3.1. Total Phenolic Content (TPC)

The results of total phenolic content (Table 3) varied from 6.77 ± 0.38 to 10.295 ± 0.29 mg GAE/g on a fresh weight basis, with Barbados cherry showing the highest TPC (10.295 mg GAE/g). There was no significant difference between the TPC values of each fruit ($p > 0.05$).

3.3.2. Antioxidant Activity (AOA)

According to Table 3, there was no significant difference between the FRAP values of each fruit ($p > 0.05$). The FRAP values varied from 0.015 ± 0.003 to 0.022 ± 0.003 Fe^{2+} mmol/g fresh fruit. A study on the antioxidant capacity and bioactive compounds of four Brazilian native fruits showed that the values for the FRAP assay ranged from 0.009 ± 0.0009 to 0.089 ± 0.013 mmol $FeSO_4 \cdot 7H_2O$/g fresh weight and the FRAP values of

fruits in our study also fell into this range. According to the values obtained for the DPPH radical scavenging capacity, the IC_{50} value of Barbados cherry (0.040 ± 0.002 mg/mL IC_{50}) was the lowest, indicating that these had the highest antioxidant activity among the tested fruits (Table 3).

3.4. Vanillin Content

The vanillin content of underutilized fruit extracts is shown in Figure 2. According to this, the Barbados cherry (2.43 mg/kg) had the highest vanillin content, followed by Uguressa (2.095 ± 0.001 mg/kg), Himbutu (1.471 ± 0.001 mg/kg), Ceylon gooseberry (1.353 ± 0.001 mg/kg), Maha Karamba (1.234 ± 0.002 mg/kg), and the lowest was observed in Màdan (0.400 ± 0.006 mg/kg).

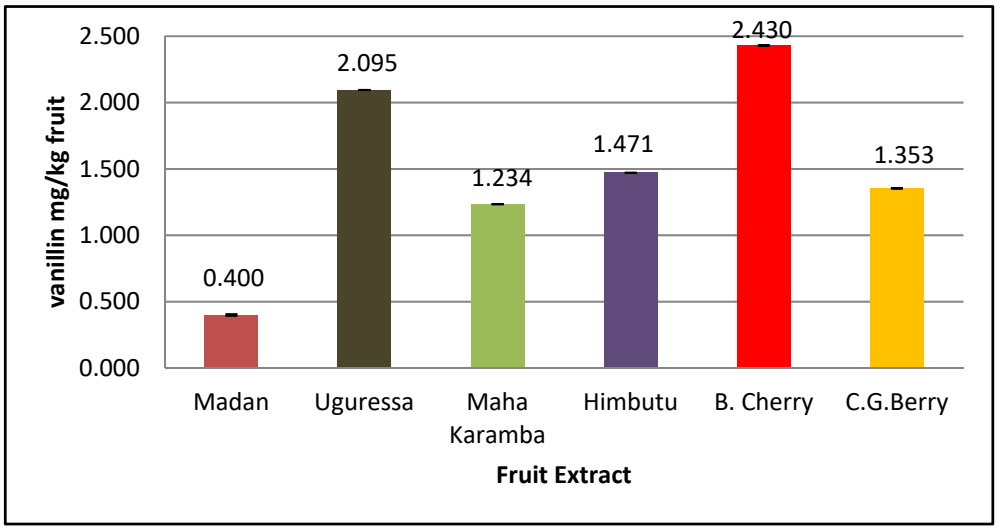

Figure 2. Vanillin content of six underutilized fruit extracts.

3.5. Mineral Content

The mineral content of underutilized fruits tested is presented in Table 4.

Table 4. Mineral content (mg/kg) of fruits studied.

Mineral	Madan	Uguressa	Maha Karamba	Himbatu	Barbados Cherry	Ceylon Gooseberry
K	149.70 ± 0.75 ^{d}	434.60 ± 0.36 ^{a}	219.20 ± 0.70 ^{b}	109.10 ± 0.36 ^{e}	150.50 ± 0.79 ^{d}	216.00 ± 1.28 ^{c}
Mg	13.25 ± 0.38 ^{a}	9.83 ± 0.74 ^{b}	8.33 ± 0.34 ^{c}	12.32 ± 0.37 ^{a}	2.10 ± 0.19 ^{d}	1.95 ± 0.23 ^{d}
Na	5.28 ± 0.30 ^{a}	3.56 ± 0.14 ^{c}	4.70 ± 0.20 ^{b}	2.86 ± 0.11 ^{d}	2.85 ± 0.15 ^{d}	2.90 ± 0.29 ^{d}
P	9.73 ± 0.11 ^{c}	16.69 ± 0.46 ^{a}	15.16 ± 0.45 ^{b}	7.03 ± 0.21 ^{e}	7.50 ± 0.49 ^{d,e}	8.50 ± 0.71 ^{c,d}
Ca	9.45 ± 0.58 ^{e}	23.43 ± 0.45 ^{a}	16.93 ± 0.41 ^{c}	14.29 ± 0.59 ^{d}	18.50 ± 0.71 ^{b}	19.00 ± 0.29 ^{b}
Fe	0.65 ± 0.12 ^{a}	0.28 ± 0.08 ^{b}	0.29 ± 0.04 ^{b}	0.15 ± 0.03 ^{b}	0.14 ± 0.04 ^{b}	0.14 ± 0.05 ^{b}
Mn	0.11 ± 0.06 ^{c}	0.47 ± 0.11 ^{b}	0.08 ± 0.03 ^{c}	0.98 ± 0.18 ^{a}	0.02 ± 0.00 ^{c}	0.02 ± 0.01 ^{c}
Al	1.15 ± 0.16 ^{a}	0.33 ± 0.04 ^{c,d}	0.54 ± 0.11 ^{b,c}	0.12 ± 0.04 ^{d}	0.65 ± 0.11 ^{b}	0.44 ± 0.05 ^{b,c}
Cu	0.07 ± 0.03 ^{a,b}	0.11 ± 0.04 ^{a}	0.11 ± 0.03 ^{a}	0.08 ± 0.03 ^{a,b}	0.03 ± 0.00 ^{b}	0.03 ± 0.00 ^{b}

Values represent the mean ± SD of triplicate analysis; n = 3. Means bearing different superscripts are significantly ($p < 0.05$) different.

According to Table 4, the highest K amount was found in Uguressa (434.6 ± 0.36 mg/kg) and the lowest content was in Himbutu (109.1 ± 0.36 mg/kg). Uguressa had a significantly high amount of K compared to all other tested fruit types ($p < 0.05$), with very high mean differences ranging from 325.5–215.4 mg/kg. The highest content of Mg was observed in Màdan (13.25 ± 0.38 mg/kg) and the lowest content in Ceylon gooseberry (1.95 ± 0.23 mg/kg) but there was no significant difference ($p > 0.05$) between Madan and Himbatu or between Barbados cherry and Ceylon Gooseberry fruits. Madan had a significantly high amount of Na when compared with other tested fruits ($p < 0.05$). The highest p was found in Uguressa

(16.69 ± 0.46 mg/kg) and this amount was significantly higher ($p < 0.05$) than other fruits with mean differences ranging from 9.66–1.53 mg/kg. The highest Ca amount also was obtained from Uguressa (23.43 ± 0.45 mg/kg) with a significantly high ($p < 0.05$) mean difference ranging from 13.98–4.43 mg/kg. The highest Fe and Al contents were found in Mãdan and both values were significantly higher ($p < 0.05$) than other tested fruits. A significantly high amount of Mn ($p < 0.05$) was reported from Himbatu (0.98 ± 0.18 mg/kg). There was no significant difference between Cu content in Uguressa and Maha Karamba ($p > 0.05$), which showed the highest amount of Cu 0.11 ± 0.04 mg/kg and 0.11 ± 0.03 mg/kg, respectively.

4. Discussion

4.1. Proximate Composition

The moisture, ash, protein, and fat content of six underutilized fruits per 100 g of fresh fruit weight (FW) were measured. In a previous study conducted by Silva et al. [37] for the six Arecaceous fruits (*Acrocomia intumescens*, *Pinanga kuhlii*, *Ptychosperma macarthurii*, *Syagrus cearensis*, *Syagrus coronata*, and *Veitchia merrillii*) the moisture level of fruit pulp ranged from 60% to 75%, except for *P. kuhlii* (22.9%), which had a lower range than the tested fruits. Another study conducted by de Souza et al. [38] reported that the moisture content varied from 86.43% (cherry) to 92.68% (strawberry), ash content varied from 0.08% (blueberry) to 0.42% (cherry), fat content varied from 0.19% (blueberry) to 0.42% (blackberry), and protein content varied from 0.48% (blueberry) to 1.27% (blackberry). When compared, the tested fruits in our study had a lower moisture content, higher ash content, higher fat content, and a similar range of protein content.

4.2. Total Dietary Fiber (TDF) Content

Dietary fiber can generally be explained as the carbohydrate content of food that is not digested in the human small intestine and which passes into the large intestine, where it is partially or fully fermented. Total dietary fiber analysis involves the determination of total fiber in cereals, beans, vegetables, and fruits. These characteristics of dietary fiber have numerous health benefits. In the study conducted by de Souza et al. [38] for berry fruits, the TDF content varied from 1.90 (blackberry) to 4.47% (blueberry) for fresh weight. The TDF content of the tested fruits in our study was higher than the TDF range of the abovementioned study. The fiber content of seven types of fruits tested in a study by Marlett and Vollendorf [39] varied from 0.3% to 8.0% fresh weight. The total fiber content of 25 fruits tested in a study by Dreher [40] varied from 0.4% to 6.8% fresh weight. Maha karamba and Uguressa showed a higher TDF content compared to these values. Food which contains more than 5 g of fiber per serving is considered high-fiber food [39]. Therefore, the underutilized fruits of our study can be categorized as high-fiber foods when considering 100 g as the serving size.

4.3. Total Phenolic Content (TPC) and Antioxidant Activity (FRAP and DPPH)

4.3.1. Total Phenolic Content (TPC)

Song et al. [41] investigated the TPC of 56 selected medicinal plant species and showed that the values varied from 0.12 to 59.43 mg GAE/g of plant parts. The TPC values obtained for fruits in this study were within the above range. The total phenolic content of extensively used medicinal plants in Sri Lanka was studied by Jayathilake et al. [42], where TPC values varied from 1.87 ± 0.43 to 5.22 ± 0.06 mg GAE/ g of plant parts, except for *Phyllanthus emblica* (Nelli). This indicates that the TPC values obtained for all six fruits in our study were higher than those of the medicinal plants [42]. A study conducted to determine the chemical composition of mulberry fruit varieties showed that the TPC value of mulberries varied from 181 to 1035 mg GAE/ g of fresh weight [43]. This range is significantly higher than the TPC values of the fruits tested in our study. In a study of berries by de Souza et al. [38], TPC ranged from 3.05 ± 0.05 (blueberry) to 8.5 ± 0.05 mg GAE/g (blackberry) of fresh weight, which was comparatively lower than the values of Màdan, Himbutu, and Barbados cherry in our study.

4.3.2. Antioxidant Activity (AOA)

The antioxidant activity of Red pitanga (0.02343 ± 0.0044 mmol $FeSO_4 \cdot 7H_2O$/g FW) had a similar value to the tested fruits [44]. In a similar study on the antioxidant activities of peel, pulp, and seed fractions of twenty-eight common fruits, the antioxidant values (FRAP assay) of fruit pulp varied from 0.14 ± 0.03 to 13.42 ± 0.74 mmol/g on a wet weight basis, which was a higher range than that of the tested fruits. Among these twenty-eight fruits, Lukan tangerine, honey tangerine, orange, and lemon showed FRAP values of 2.29 ± 0.13, 2.19 ± 0.08, 1.89 ± 0.19, and 1.43 ± 0.07 mmol/g on wet weight basis, respectively [45].

The IC_{50} values of 20 medicinal plant extracts used in Ayurvedic medicine varied from 19.48 ± 1.01 to 245.68 ± 2.01 µg/mL [46]. The IC_{50} values of the tested fruit extracts (40.0 ± 2 to 89.0 ± 1 µg/mL) were within the range of the medicinal plants, which indicates that our tested fruits showed antioxidant activity in the same range as the medicinal plants. In a study carried out on the antioxidant activity of fruits and vegetables in Algeria [47] the IC_{50} values measured via the DPPH assay ranged from 0.32 ± 0.01 (pomegranate) to 9.20 ± 0.87 (banana) mg/mL, with higher IC_{50} values but lower antioxidant activity than the tested fruits. In a similar study carried out to determine the antioxidant activities of berries [48], the IC_{50} values for DPPH radical scavenging activity were as follows: blueberry, 0.70 mg/mL; raspberry, 0.80 mg/mL; blackberry, 1.40 mg/mL; and strawberry. 5.60 mg/mL, thus showing lower antioxidant activity than the fruits tested in the present study (0.040 ± 0.002 to 0. 089 ± 0.001).

4.4. Vanillin Content

Vanillin is well-known phenylpropanoid (4-hydroxy-3-methoxybenzaldehyde) that is mainly used as a flavoring agent/additive to foods and beverages and as an intermediate for various pharmaceuticals [19]. In a study on the determination of vanillin in several fruit extracts using GC-olfactometry and GC-MS/MS, the vanillin content in orange, tangerine, lemon, and lime were 0.20, 0.35, 0.41, and 0.35 mg/kg, respectively, which was significantly lower than the vanillin content in the tested fruits, except for Màdan (0.4 mg/kg) which had a similar vanillin content to the citrus fruits in the above study [16].

In this study, the most appropriate flow rate was found to be 0.4 mL/min. The main use of vanillin is for flavor enhancement, and it is used abundantly in the food industry [18]. The health benefits of vanillin can be attributed to its antioxidant, aphrodisiac, anti-carcinogenic, anti-depressant, sedative, tranquilizing, and relaxing properties [49]. Therefore, the quantification of vanillin has been routinely performed for many plant materials in the past. Therefore, this analysis is highly important since the consumption of these fruits can provide many beneficial properties. It has been reported that the effect of various extraction parameters, such as type of the solvent, the number of beans, and the method of extraction (Soxhlet extraction and ultrasound-assisted extraction), influence the content of vanillin obtained from cured vanilla beans [50]. In this study, extraction was carried out with 70% methanol.

4.5. Mineral Content

Inductively coupled plasma mass spectrometry (ICP-MS) is an elemental analysis technology. The ICP source converts the atoms of the elements in the sample to ions, and these ions are then separated and detected by the mass spectrometer. ICP-MS is capable of scanning the mass-to-charge (m/z) range of 5 to 240 amu, with a minimum resolution of 0.9 amu at 10% peak height, and a mass flow controller for nebulizer gas. The metal elements detected by means of ICP-MS are Na, P, K, Ca, Mg, Fe, Al, Mn, and Cu. When a specific element to be detected is present in a higher concentration, they are diluted by dilution factors of 10, 20, or 250.

Minerals act as electrolytes in the human body. Important electrolytes for physiological functions in humans include sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), and chloride (Cl^-). As a group, these electrolytes are involved in countless activities that are essential for life, including energy production, nerve transmission, muscle contractions, pH

balance, fluid balance, and more. The human body, which is a remarkable self-regulating organism, has a number of mechanisms in place to maintain a proper electrolyte balance [51]. Therefore, minerals which act as electrolytes must be included in the daily diet. This study revealed that Màdan is high in Na and Mg, whereas Uguressa is rich in K and these fruits can be consumed in order to meet the daily mineral needs of the body.

5. Conclusions

Of the six underutilized fruits studied, Ugurassa showed the highest dietary fiber content. Barbados cherry showed the highest antioxidant efficacy, as measured by TPC, FRAP and IC_{50} values. The underutilized fruits tested are rich sources of K, Ca, and P. Despite their richness in nutrients and antioxidant activity, these fruits remain underutilized in Sri Lanka. They are not commercially exploited, nor are they available for consumers, although they are grown in small quantities in some areas of the country. The present study will shed light on the hidden value of some of these underutilized fruits grown in this country and this will promote their utilization.

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