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Phytochemicals, Functional and Physiochemical Properties of Selected Unexploited Fruits in Sri Lanka

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PHYTOCHEMICALS, FUNCTIONAL AND PHYSIOCHEMICAL PROPERTIES OF SELECTED UNEXPLOITED FRUITS IN SRI LANKA

Abstract: As a tropical country with high biodiversity, Sri Lanka has a diversified variety of fruits, however most of them remain unexploited. Unfortunately, most Sri Lankans are not aware of those neglected fruit crops and their nutritional value and health benefits. Therefore, this study was carried out to determine the total antioxidant capacity, bioactive compounds and physiochemical properties of nine selected underutilized fruit species in Sri Lanka. Extracts of selected nine unexploited fruits namely, *Syzygium cumini* (Java plum), *Ardisia elliptica* (Shoebutton fruit), *Eugenia uniflora* (Surinam cherry), *Antidesma alexiteria* (Ceylon bignay), *Dovyalis hebecarpa* (Ceylon gooseberry), *Malpighia emarginata* (Barbados cherry), *Morus alba* (Mulberry), *Flacourtia indica* (Governor's plum) and *Antidesma bunius* (Bignay) were assessed for their total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC) as well as physiochemical properties. TPC, TFC and TAC were determined by Folin-ciocalteu method, a colourimetric method and ferric reducing antioxidant power (FRAP) assay respectively. The fresh weight (FW), moisture content and total soluble solid (TSS) were also determined. Statistical Analysis was done by analysis of variance (ANOVA) followed by Tukey Multiple Range Test using SAS. The highest fresh weight was observed in *Eugenia uniflora* (7.31 ± 0.60 g) whereas, the highest moisture content was recorded in *Morus alba* (88.81 ± 0.48 %). *Flacourtica indica* had the highest TSS value (19.95 ± 0.71%). *Malpighia emarginata* had significantly the highest TAC (2623 ± 43.37 mg TE/100 g FW) and TPC (1624.01 ± 71.45 mg GAE/100 g FW) whereas, significantly high TFCs were observed in *Flacourtica indica* (1374.03 ± 222.61 mg RE/100 g FW) and *Syzygium cumini* (1305.97 ± 202.17 mg RE/100 g FW). This study concluded that *Malpighia emarginata*, *Syzygium cumini* and *Flacourtica indica* had potent sources of bioactive compounds and antioxidants.

Keywords: Antioxidant capacity, Flavonoids, Phenolics, Unexploited fruits

Introduction

As a tropical country with high biodiversity, Sri Lanka has a diversified variety of fruits (Abeysuriya *et al*., 2020; Perera *et al*., 2022). There are more than 230 fruit species belonging to 57 plant families produced in Sri Lanka (Piyathunga *et al*., 2016). However, most of fruit species remain underutilized or unexploited mainly which are indigenous to Sri Lanka. Fruit crops that are not frequently consumed by people and not cultivated commercially are referred to as underutilized fruits (Dahanayake, 2015). They are only present as small quantities at domestic level. The entire nation is primarily dependent on a small number of crop species; few vegetables, fruits and other crops as a result of the commercialization by ignoring a significant number of vegetables, fruits, and other crops (Dahanayake, 2015). There are around 60 underutilized fruit species in Sri Lanka (Dahanayake, 2015; Ratnayake *et al*., 2019).

The damaging intermediates known as reactive oxygen species (ROS) are created during the oxygen metabolism in biological systems. Oxidative stress is a situation brought on by an excess of ROS in the body, which can result in cumulative damage to proteins, lipids, and DNA (Mallawaarachchi *et al*., 2019). Age-related disorders, cancer, cardiovascular, inflammatory, and neurodegenerative diseases including Parkinson's and Alzheimer's disease are only a few of the pathologies linked to oxidative stress. A diet rich with fruits and vegetables is linked to a lower risk of death from chronic diseases according to epidemiological studies (Perera *et al*., 2022). Numerous neglected fruits have high bioactive compounds, antioxidants, anti-cancer, and antibacterial components in addition to being excellent sources of fiber, vitamins, and minerals (Perera *et al*., 2022; Hettiarachchi and Gunathilake, 2020). Due to their abundance in useful bioactive compounds, tropical fruits are regarded as one of the potential sources of antioxidants. Sri Lanka's daily per capita consumption of fruit is around 40 g for a healthier diet, but it remains at a lower level (Dahanayake, 2015). Unfortunately, most Sri Lankans are not aware of those neglected fruit crops and their nutritional value and health benefits. Even without knowledge of taste or nutritional value, the majority of Sri Lanka's edible fruits continue to go largely unnoticed or unutilized (Abeysuriya *et al.,* 2020).

Therefore, this study was carried out to determine the total antioxidant capacity, bioactive compounds and physiochemical properties of nine selected underutilized fruit species in Sri Lanka.

Methodology

Location

The study was conducted at the laboratory within the Department of Plantation Management, located in the Faculty of Agriculture and Plantation Management at Wayamba University of Sri Lanka in Makandura, Gonawila (NWP).

Sample Collection

Nine (09) unexploited fruits which are shown in the Table 1 were used in this study. The selected unexploited fruits were harvested freshly from naturally grown trees in different locations and from selected home gardens in Sri Lanka at ripen stage. Harvested fruits were transported to the laboratory under cool conditions (0^0C-4 ^{0}C).

Preparation of Fruit Samples

The harvested fresh fruits were combined to obtain the composite sample. The fruits were washed to remove dirt particles on the skin of the fruits and edible portion was used to prepare extracts. Extractions were done in triplicates.

Table 1. Information of selected fruit species

No	Botanical name	Common name (s)	Edible part	Harvested location	Harvested season month/year
1	*Syzygium cumini* L	Java Plum Fruit, *Ma Dan*	FWS	*Palawaththa* (WL_1)	11/2022
2	*Ardisia elliptica thunb*	Shoebutton Fruit, *Balu Dan*	WF	*Dharga Town* (WL_1)	12/2022
3	*Eugenia uniflora*	Surinam Cherry, *Cheena Goraka*	FWS	*Dharga Town* (WL_1)	12/2022
4	*Antidesma alexiteria*	Ceylon Bignay, *Heen Ambilla,*	WF	*Dankotuwa* (IL_1)	12/2022
5	*Dovyalis hebecarpa*	Ceylon Goose Berry, *Puss Berry*	FWS	*Dankotuwa* (IL_1)	12/2022

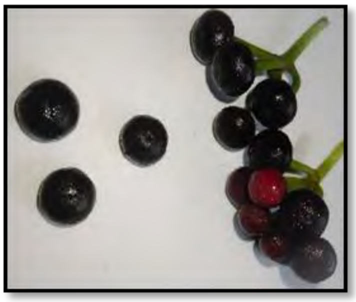
6	*Malpighia emarginata*	Barbados Cherry, Garden Cherry	FWS	*Dankotuwa* (IL_1), Dharga *Town* (WL_1)	01/2023
7	*Morus alba*	Mulberry*, Rata Ambilla*	WF	*Makandur*a (IL_1)	01/2023
8	*Flacourtia indica*	Governor's Plum, *Uguressa*	WF	*Dankotuwa* (IL_1)	02/2023
9	*Antidesma bunius*	Bignay, *Karawala Kebilla*	FWS	*Dankotuwa* (IL_1)	01/2023

FWS- Fruit without seed(s), WF- Whole fruit, WL- Low Country Wet Zone, IL- Low Country Intermediate Zone

Determination of Physiochemical Properties

Fresh weight, peel colour and flesh colour of each fruit species were recorded separately by using Royal Horticultural Society Colour Chart. The samples' moisture content was assessed by drying them at 80 ± 2 °C until a consistent weight was achieved. Regarding a chemical characteristic, the total soluble solid content (TSS) of the fruit juice was measured using a digital hand refractometer. (HR-120, Italy).

A



B

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I

Figure 1. Collected samples of selected unexploited fruits

(A) Java plum fruit (B) Shoebutton fruit (C) Surinam cherry (D) Ceylon bignay (E) Ceylon gooseberry (F) Barbados cherry (G) Mulberry (H) Governor's plum (I) Bignay

Chemicals and Reagents

Folin-Ciocalteu reagent, Gallic acid, Ferric chloride (FeCl. $6H_2O$), 2,4,6-tripyridyl-2-try-azine (TPTZ), 6-hydroxy-2,5,7,8-tetra methyl-chroman-2-carboxilic acid (Trolox) and Rutin were purchased from Sigma Aldrich Chemical Co. (St. Louis, Mo). All other chemicals were used in analytical grade

Methanolic Extraction of Samples

The fresh fruit sample was weighed (2 g) and ground using motor and pestle with the addition of chilled 80% methanol. Next, the samples underwent homogenization using a homogenizer (Witeg, 0400189139T002, German) for a duration of 2 minutes. The resulting homogenate was then mixed with chilled 80% methanol, reaching a final volume of 50 ml. The fruit extracts were transferred to 15 ml Falcon tubes and centrifuged at 4,000 rpm for 5 minutes to eliminate the solid fraction. Subsequently, the extracted samples were preserved at -20°C until further analysis.

Determination of Total Phenolic Content (TPC)

Total phenolic contents of the fruit extracts were determined using Folin-ciocalteu method as described by Abeysinghe *et al.* (2007). Briefly, 4 ml of distilled water and 0.5 ml of properly diluted fruit extract were placed in a 15 ml falcon tubes. To the reaction mixture, 0.5 ml of Folin-ciocalteu reagent was introduced and allowed to react for a duration of 3 minutes. Subsequently, 1 ml of saturated sodium carbonate solution was added, and the samples were incubated in a water bath at 30°C for 2 hours. The absorbance was then measured at 760 nm using a UV-visible spectrophotometer (Shimadzu, UV Mini 1240, Japan). Gallic acid served as the standard, and the data were expressed as milligrams of gallic acid equivalents (GAE) per 100 grams of fresh weight (FW).

Determination of Total Flavonoid Content (TFC)

Total flavonoid content (TFC) was determined by the colourimetric method with slight modifications (Liu *et al*., 2002). A volume of 0.5 ml of a known dilution of fruit extract was added to falcon tubes containing 3.5 ml of distilled water and mixed with 0.3 ml of 5% NaNO2. After 6 minutes, 0.3 ml of a 10% $A1(NO)_3.6H_2O$ solution was introduced. The mixture was left undisturbed for an additional 6 minutes, followed by the addition of 2 ml of 2M NaOH. Subsequently, the reaction mixture was diluted with 1.4 ml of distilled water, and the absorbance at 510 nm was promptly measured using a spectrophotometer (Shimadzu, UV Mini 1240, Japan). The total flavonoid content was computed utilizing the Rutin standard curve and expressed as milligrams of Rutin equivalents (RE) per 100 grams of fresh weight (FW).

Determination of Total Antioxidant Capacity (TAC)

The determination of total antioxidant capacity utilized the ferric reducing antioxidant power (FRAP) assay, following the method outlined by Benzie and Stain (1996). In brief, the FRAP reagent was freshly prepared by combining 300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ solution, and 20 mM ferric chloride solution. The absorbance at 593 nm was measured four minutes after the mixture of 100 µl of fruit extract with 900 µl of FRAP reagent, using a spectrophotometer (Shimadzu, UV Mini 1240, Japan). The total antioxidant capacity was expressed as milligrams of Trolox equivalents (TE) per 100 grams of fresh weight (FW).

Statistical Analysis

Parametric variables were analyzed using analysis of variance (ANOVA) followed by Tukey Multiple Range Test using SAS (SAS Version 9.4). Means and standard deviation were reported.

Results and Discussion

Physiochemical Properties

Fruit peel and flesh colour, fresh weight, moisture content and total soluble solid (TSS) of selected unexploited fruits are shown in Table 2 and 3. Fruit peel colour was varied from red, purple to black and fruit flesh colour was varied from orange to purple. The highest fresh fruit weight was observed in Surinam cherry (7.31 ± 0.60 g) whereas, the lowest fruit weight (0.06 ± 0.01 g) was observed in Ceylon bignay. Moisture content was varied from 70.80 ± 0.56 % (Governor's plum) to 88.81 ± 0.48 % (Mulberry). Governor's plum had the highest TSS value (19.95 ± 0.71 %) and the lowest TSS value was recorded in Barbados cherry (6.58 ± 0 .33 %).

Table 2. Peel and flesh colour of selected unexploited fruits

Fruit Species	Peel Colour	Flesh Colour
Syzygium cumini	Black Group 202-A	Purple Group 76-B
Ardisia elliptica	Black Group 202-A	Violet Group 84-C
Eugenia uniflora	Red Group 44-A	Red Group 40-A
Antidesma alexiteria	Black Group 202-A	Greyed Purple Group 185-B
Dovyalis hebecarpa	Purple Group N77-A	Greyed Orange Group 163-A
Malpighia emarginata	Greyed Purple Group 185-A	Greyed Orange Group N163-B
Morus alba	Black Group 202-A	Greyed Purple Group N-186-A
Flacourtia indica	Greyed Purple Group 183-A	Greyed Orange Group N172-D
Antidesma bunius	Black Group 202-A	Greyed Red Group 179-D

Table 3. Physiochemical properties of selected unexploited fruits

Fruit Species	Fresh Weight (g)	Moisture (%)	TSS (Brix %)
Syzygium cumini	2.85 ± 0.18^{d}	78.22 ± 1.07^{c}	10.71 ± 0.78^{c}
Ardisia elliptica	0.29 ± 0.03^{fg}	72.74 ± 0.89^{c}	12.91 ± 0.35^{b}

Eugenia uniflora	7.31 ± 0.60[a]	86.44 ± 1.16[ab]	9.19±0.89[d]
Antidesma alexiteria	0.06 ± 0.01[g]	76.70 ± 0.79[c]	12.65±0.74[b]
Dovyalis hebecarpa	6.87 ± 0.52[a]	88.33 ± 0.07[ab]	9.59±0.64[d]
Malpighia emarginata	3.71 ± 0.35[c]	86.29 ± 0.27[b]	6.58±0.33[f]
Morus alba	1.52 ± 0.25[e]	88.81 ± 0.48[a]	13.12±0.79[b]
Flacourtia indica	4.67 ± 0.47[b]	70.80 ± 0.56[d]	19.95±0.71[a]
Antidesma bunius	0.64 ± 0.06[f]	86.85 ± 1.54[ab]	7.54±0.22[e]

Means denoted by the same letters in a column represent non-significant differences (p<0.05); TSS= Total Soluble Solid

Functional Properties

TPC content was varied from 107.34 ± 3.96 to 1,624.01 ± 71.45 mg GAE/100 g of FW (Table 4). Among all fruit species tested, Barbados cherry had significantly highest TPC (1,624.01 ± 71.45 mg GAE/100 g of FW). The similar results have reported by Perera *et al.* (2022). There was no significant variation in the TPC between Surinam cherry, Ceylon gooseberry and bignay which had the lowest TPC values. In a study of 24 commercial fruit species reported by Wu *et al.* (2004), TPC ranged from 368 ± 80 mg GAE/100 g of FW (Strawberry) to 660 ± 285 mg GAE/100 g of FW (Blackberry), which was comparatively lower than the TPC values of Barbados cherry (1,624.01 ± 71.45 mg GAE / 100 g of FW), Java plum (944.84 ± 18.46 mg GAE/100 g of FW) and governor's plum (726.98 ± 27.57 mg GAE/100 g of FW) in our study.

There was a significant variation in TFC of all selected unexploited fruit species (Table 4). High TFCs were observed in governor's plum (1,374.03 ± 222.61 mg RE/100 g of FW) and Java plum (1,305.97 ± 202.17 mg RE/100 g of FW) whereas, Ceylon gooseberry reported the lowest TFC value (1.19 ± 0.48 mg RE/100 g of FW). However, no significant difference was observed in TFCs between Ceylon gooseberry, Surinam cherry, bignay and Ceylon bignay. In the study of two dragon fruit species grown in Sri Lanka reported by Senadheera and Abeysinghe (2015), TFC of red flesh (46.29 ± 2.47 mg RE/100 g of FW) and white flesh (26.71 ± 4.46 mg RE/100 g of FW) of dragon fruit was comparatively lower than the TFC values of governor's plum, Java plum, mulberry, shoebutton fruit, Barbados cherry and Ceylon bignay in our study (Table 4).

The total antioxidant capacity (TAC) varied significantly among selected nine unexploited fruit species (Table 4). Among all species tested, Barbados cherry had significantly highest TAC (2,622.74 ± 43.37 mg TE/100 g of FW) followed by Java plum (2,050.08 ± 210.60 mg TE/100 g of FW) and governor's plum (1,140 ± 201.66 mg TE/100 g of FW). The results obtained in this study agreed with the findings of Perera *et al.* (2022). Ceylon gooseberry recorded the lowest TAC (66.89 ± 9.76 mg TE/100 g of FW); however, it was not significantly different from the TAC values of Surinam cherry (128.23 ± 2.83 mg TE/100 g of FW) and Bignay (167.02 ± 34.58 mg TE/100 g of FW). In a study of 14 dessert type banana accessions in Sri Lanka reported by Hettiarachchi *et al*. (2018), TAC of banana pulp ranged from 5.23 ± 0.4 mg TE/100 g of FW (*Suwandel*) to 94.66 ± 2.7 mg TE/100 g of FW (*Puwalu*), which was comparatively lower than the TAC values of all the selected unexploited fruits in our study (Table 4). Another study of thirty citrus varieties grown in Sri Lanka was reported by Jayawardhana *et al*. (2018), TAC of juice filled vesicles of citrus ranged from 12.99 ± 1.50 TE/100 g of FW (Lemon) to 554.77 ± 2.26 TE/100 g of FW (Sweet orange *Sisila*), which was comparatively lower than the TAC values of all the selected unexploited fruits in our study (Table 4).

The present analysis showed a significantly strong correlation ($R^2 = 0.924$) between TAC and TPC of selected unexploited fruits. This strong correlation confirmed that phenolic constituents of fruit extracts represent a major contribution to the fruit's antioxidant activity. However, poor correlation between TAC and TFC ($R^2 = 0.312$) indicating that TFC make a very low contribution to the fruit's TAC as compared to the total phenolic content.

Table 4. total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC) of selected unexploited fruit species

Fruit species	**TPC (mg GAE/100 g FW)**	**TFC (mg RE/100 g FW)**	**TAC (mg TE/100 g FW)**
Syzygium cumini	944.84 ± 18.46^{b}	1305.97 ± 202.17^{a}	2050.08 ± 210.60^{b}
Ardisia elliptica	358.33 ± 20.11^{d}	601.81 ± 36.40^{b}	987.18 ± 76.21^{cd}
Eugenia uniflora	107.34 ± 3.96^{e}	22.58 ± 1.44^{d}	128.23 ± 2.83^{e}
Antidesma alexiteria	353.77 ± 0.13^{d}	207.36 ± 4.81^{cd}	877.50 ± 38.94^{cd}
Dovyalis hebecarpa	79.56 ± 8.55^{e}	1.19 ± 0.48^{d}	66.89 ± 9.76^{e}
Malpighia emarginata	1624.01 ± 71.45^{a}	400.42 ± 12.50^{bc}	2622.74 ± 43.37^{a}
Morus alba	339.68 ± 25.46^{d}	614.31 ± 33.94^{b}	675.08 ± 174.67^{d}
Flacourtia indica	726.98 ± 27.57^{c}	1374.03 ± 222.61^{a}	1140.48 ± 201.66^{c}
Antidesma bunius	155.36 ± 3.31^{e}	30.97 ± 4.81^{d}	167.02 ± 34.58^{e}

Means denoted by the same letters in a column represent non-significant differences ($p<0.05$); TE - Trolox Equivalents; GAE - Gallic Acid Equivalent; RE - Rutin Equivalent; FW-Fresh Weight

Conclusions

This study revealed that among selected nine unexploited fruit species, Barbados cherry (TPC: 1624.01 mg GAE/100 g FW, TFC: 400.42 mg RE/100 g FW, TAC: 2622.74 mg TE/100 g FW) Java plum (TPC: 944.84 mg GAE/100 g FW, TFC: 1305.97 mg RE/100 g FW, TAC: 2050.08 mg TE/100 g FW) and governor's plum (TPC: 726.98 mg GAE/100 g FW, TFC: 1374.03 mg RE/100 g FW, TAC: 1140.48 mg TE/100 g FW) fruits had significantly higher TPC, TFC and TAC whereas, low levels of bioactive compounds and total antioxidant capacity were recorded in Surinam cherry, Ceylon gooseberry and bignay fruits.

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