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Research Article

Phytochemicals, proximate composition, and antioxidant properties of selected underutilized fruits in Sri Lanka

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Abstract: This research focused on the phytochemical and proximate analysis, as well as the antioxidant properties of 10 underutilized fruit species found in Sri Lanka. The study qualitatively tested the presence of phytochemicals; specifically polyphenols, flavonoids, tannins, and saponins, in various fruit extracts using methanol, water, and acetone as solvents. The total phenolic and flavonoid contents were quantified using the Folin-Ciocalteu and aluminum chloride methods, respectively. Antioxidant activity was evaluated through 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and Ferric Reducing Antioxidant Power (FRAP) assays. Additionally, the fruits were analyzed for vitamin C, fat, protein, carbohydrate, moisture, and ash content. The results indicated that the total phenolic and flavonoid contents, as well as antioxidant activities, varied based on the extraction solvent used, with acetone proving to be the most effective for extracting these compounds. *Phoenix pussilla* and *Syzygium caryophyllatum* exhibited the highest levels of total phenolics, flavonoids, and ascorbic acid. Moreover, *P. pussilla*, *Antidesma ghaesembilla*, *Antidesma alexiteria*, and *S. caryophyllatum* demonstrated significant antioxidant properties. The findings of this research highlight the potential of the 10 selected underutilized fruits and suggest enhancing their commercial value while supporting biodiversity conservation strategies.

1. INTRODUCTION

Sri Lanka, a tropical country in South Asia, is home to a diverse range of indigenous edible fruits with significant nutritional benefits. These fruits have been a staple in the local diet. However, they have become increasingly overlooked due to changing dietary habits, evolving lifestyles, and a lack of confirmed information about their nutritional qualities. Wild fruits, which grow naturally and are neither cultivated nor commercialized, have received less scientific attention compared to commercially available fruits.

Fruits generally contain more antioxidants than vegetables, pulses, and cereals, with their antioxidant properties largely attributed to their phenolic compound content. While there is extensive information on the antioxidant activities and phytochemical compositions of commercial fruits, knowledge about tropical underutilized fruits remains relatively limited (Lamien-Meda *et al.*, 2008; Soong & Barlow 2004). Antioxidants are crucial for protecting the

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body from oxidative stress and free radical damage, playing an essential role in maintaining overall health. The effectiveness of antioxidants is measured by their reduction potential, which indicates their ability to donate electrons and form stable radicals (Rajashekar *et al.*, 2009). Key antioxidants include vitamins C and E, beta-carotene, selenium, and various phytochemicals found in fruits, vegetables, and other plant-based foods (Myhrstad & Wolk, 2023).

Studies have suggested that wild fruits may offer higher nutritional value compared to cultivated varieties (Stadlmayr *et al.*, 2013). Proximate analysis, which evaluates macronutrients such as moisture, ash, crude fiber, crude fat, crude protein, and carbohydrates, is a standard method for assessing food quality (Untalan *et al.*, 2015). Moisture content is crucial for food quality and shelf life, as fruits with lower moisture are less prone to microbial degradation and tend to have longer shelf lives (Aruah *et al.*, 2012). Additionally, the physical properties of fruits such as size, viscosity, weight, and bulk density, are influenced by moisture content, impacting harvesting, transportation, storage, and processing (Hegazy *et al.*, 2019). Ash content reveals the total mineral content, including essential minerals like calcium, sodium, potassium, and chloride, offering insights into the mineral composition of the fruits (Kalsum & Mirfat, 2014).

Wild tropical fruits are renowned for their high nutritional and medicinal value and are commonly used in traditional therapeutic practices (Aguilera & Toledo, 2024; Meena *et al.*, 2022). Ongoing research into the phytochemical properties of these underutilized fruits is essential for understanding their potential health benefits and encouraging their consumption. This study aimed to evaluate the antioxidant properties and nutrient contents of 10 selected underutilized fruits in Sri Lanka to promote their consumption.

Syzygium caryophyllatum (L.) Alston (vernacular name, S: Dan) is native to Sri Lanka and India and belongs to the Myrtaceae family. This small tree predominantly grows in seasonally dry tropical regions and produces purple, succulent, edible fruits with a sweet, slightly astringent flavor (Dassanayake, 1980a). *Microcos paniculata* L. (vernacular name, S: Kohu Kirilla) belongs to the Malvaceae family. This shrub or tree is native to Sri Lanka, India, Pakistan, China, and Malaysia. It is commonly found in woodlands, small forests, forest edges, and homesteads. The plant produces edible fruits (Plants of the World Online, Kew Royal Botanic Gardens). *Antidesma ghaesembilla* Gaertn. (vernacular name, S: Bu embilla) belongs to the Phyllanthaceae family. Native to Sri Lanka, tropical and subtropical Asia, and northern Australia, this shrub or small tree primarily grows in wet tropical regions. Its fruits are deep purplish-black when fresh, sour, and edible (Plants of the World Online, Kew Royal Botanic Gardens). *Antidesma alexiteria* L. (vernacular name, S: Heen embilla) belongs to the Phyllanthaceae family. Native to Sri Lanka and South India, it is primarily found in the wet tropical biome. This shrub or small tree produces small, ovoid-oblique, red fruits (Plants of the World Online, Kew Royal Botanic Gardens). *Baccaurea motleyana* (Müll.Arg.) Müll.Arg. (vernacular name, S: Gaduguda), belongs to the Phyllanthaceae family. Native to Thailand and West Malaysia, it is considered exotic in Sri Lanka. This tree primarily grows in the wet tropical biome and bears globose to ellipsoid, 3-seeded berries (Plants of the World Online, Kew Royal Botanic Gardens).

Cynometra cauliflora L. (vernacular name, S: Namnam) belongs to the Fabaceae family. Native to Sri Lanka, South Myanmar, and West and Central Malaysia, this shrub or tree primarily grows in the wet tropical biome. Its fruits are fleshy and glabrescent (Dassanayake, 1980b). *Phoenix pusilla* Gaertn. (vernacular name, S: Idi, E: Ceylon date palm) belongs to the Arecaceae family. Native to Sri Lanka and South India, this shrub or tree primarily grows in the seasonally dry tropical biome. It is a solitary or clustering palm, producing ovoid fruits that ripen from green to red to purple-black. The fruits are moderately fleshy and sweet (Dassanayake, 2000). *Psidium guineense* Sw. (vernacular name, S: Ambul Pera) belongs to the Myrtaceae family. Native to Mexico and Tropical America, it is exotic and naturalized in Sri Lanka. This shrub or tree primarily grows in the seasonally dry tropical biome and bears

depressed globose fruits (Dassanayake, 1980a). *Ziziphus oenopolia* (L.) Mill. (vernacular name, S: Hin Eraminiya) belongs to the Rhamnaceae family. Native to Sri Lanka, China, Tropical Asia, and Northern Australia, it is a scrambling shrub or tree that primarily grows in the seasonally dry tropical biome. The fruit is a drupe, globose or ovoid, shining black when ripe, with an acrid pulp (Dassanayake, 1996). *Elaeocarpus angustifolius* Blume (vernacular name, S: Nil veralu, E: Blue marble tree) belongs to the Elaeocarpaceae family. Native to China, Malaysia, and the Southwest Pacific, it is exotic in Sri Lanka. This tree primarily grows in the wet tropical biome and produces metallic blue fruits (Plants of the World Online, Kew Royal Botanic Gardens).

2. MATERIALS and METHODS

2.1. Plant Materials and Collection

The fruit species for the research were chosen based on a reconnaissance survey conducted in Sri Lanka. Ripe fruits of the following species were collected from their natural habitats in the Veyangoda Region (7.1541° N, 80.0594° E) of the Gampaha district, Western Province, Sri Lanka: *Syzygium caryophyllatum* (L.) Alston, *Microcos paniculata* L., *Antidesma ghaesembilla* Gaertn., *Antidesma alexiteria* L., *Baccaurea motleyana* (Müll.Arg.) Müll.Arg., *Cynometra cauliflora* L., *Phoenix pusilla* Gaertn., *Psidium guineense* Sw., *Ziziphus oenopolia* (L.) Mill. and *Elaeocarpus angustifolius* Blume. The fruits were transported to the Department of Plant and Molecular Biology, University of Kelaniya, Sri Lanka, and stored at -80 °C for further analysis.

2.2. Chemicals and Reagents

Folin-Ciocalteu's Phenol reagent (Sisco Research Laboratories, India), Gallic acid (HiMedia Laboratories, India), Aluminum chloride (Sisco Research Laboratories, India), Quercetin (HiMedia Laboratories, India), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, USA), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Sigma-Aldrich, USA), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonate (ABTS) (MP-Biomedicals, USA), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (Sigma-Aldrich, USA).

2.3. Preparation of Fruit Extracts

For each fruit species, 5.0 g of the edible portion was homogenized separately in 25 mL of 70% methanol (v/v), water, and 60% acetone (v/v). The mixtures were allowed to stand overnight and then centrifuged at 5300 rpm for 10 minutes.

2.4. Qualitative Phytochemical Screening of the Fruit Extracts

Preliminary screening of phytochemicals, specifically polyphenols, flavonoids, tannins, saponins, and alkaloids, was conducted using methanolic, water, and acetone extracts from the 10 selected fruits, following established procedures (Bhandary *et al.*, 2012; Dewi & Purwayantie 2019).

2.5. Total Phenolic Content of the Fresh Fruit Extracts

The total phenolic content of the fruit extracts was determined using the Folin-Ciocalteu reagent assay (Horszwald & Andlauer, 2011). A 30 µL aliquot of each diluted fruit extract was placed in the wells of microplates. To each well, 240 µL of Folin-Ciocalteu's Phenol reagent (1:15, v/v) was added, and the mixture was incubated in the dark at room temperature (25 ± 2 ^{o}C) for 10 minutes. Following this, 30 µL of 20% sodium carbonate (Na_2CO_3) was added to each well and mixed by shaking. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Thermo Fisher Scientific, Finland). The results were expressed as milligrams of gallic acid equivalents per gram of fresh sample (mg GAE/g FW). Data analysis was performed using one-way ANOVA and Tukey's pairwise comparison via SPSS software (Chicago, USA).

2.6. Total Flavonoid Content of the Fresh Fruit Extracts

Total flavonoid content was measured following the method described by Horszwald & Andlauer (2011). To 100 µL of each fruit extract (methanol, water, and acetone), 100 µL of 10% aluminum chloride ($AlCl_3$) was added. Subsequently, 100 µL of 1 mM potassium acetate (CH_3COOK) was added. The mixture was then kept in the dark for 40-45 minutes. Absorbance was recorded at 415 nm using the UV-Vis spectrophotometer. The results were expressed as milligrams of quercetin equivalents per gram of fresh sample (mg QE/g FW). Statistical analysis was performed using one-way ANOVA and Tukey's pairwise comparison via SPSS software.

2.7. Trolox Equivalent Antioxidant Capacity (TEAC) by DPPH Assay

The free radical scavenging activity of the fruit extracts was assessed using the DPPH assay, as described by Horszwald & Andlauer (2011). To 20 µL of each fruit extract (methanol, water, and acetone), 300 µL of freshly prepared DPPH solution was added. The mixture was incubated in the dark for 30 minutes. Absorbance was measured at 517 nm, and the percentage of DPPH free radical scavenging activity was calculated. Results were expressed as micromoles of Trolox equivalents per gram of fresh sample (TE/g FW). Statistical analysis was performed using one-way ANOVA and Tukey's pairwise comparison with SPSS software.

$$\text{DPPH free radical scavenging activity (\%)} = \{(Ac - As) / Ac\} \times 100$$

Where Ac is the absorbance of the DPPH solution without extract, and As is the absorbance of the DPPH solution containing the fruit extract.

2.8. Ferric Reducing Antioxidant Potential (Frap)

FRAP was assessed following the method outlined by Benzie & Strain (1996). Freshly prepared FRAP reagent (300 µL) was warmed to 37 °C and mixed with 40 µL of each fruit extract (methanol, water, and acetone). The mixture was incubated in the dark for 10 minutes. Absorbance was measured at 593 nm using the UV-Vis spectrophotometer. The results were expressed as micromoles of $FeSO_4$ per gram of fresh weight (µmol $FeSO_4$/g FW). Statistical analysis was performed using one-way ANOVA and Tukey's pairwise comparison with SPSS software.

2.9. Trolox Equivalent Antioxidant Capacity (TEAC) by ABTS Assay

TEAC was determined using the ABTS cation radical scavenging assay, as described by Horszwald & Andlauer (2011). In this assay, 290 µL of ABTS solution was mixed with 10 µL of fruit extracts prepared in methanol, water, and acetone. The mixture was then incubated in the dark for 6 minutes. The absorbance was measured at 734 nm to assess the ABTS free radical scavenging activity. Results were expressed as micromoles of Trolox Equivalents (TE) per gram of fresh sample (TE/g FW). Statistical analysis was performed using one-way ANOVA and Tukey's pairwise comparison in SPSS software.

$$\text{ABTS free radical scavenging activity \%} = \{(Ac - As)/Ac\} \times 100$$

Where Ac represents the absorbance of the control, and As represents the absorbance of the sample.

2.10. Determination of the Vitamin C (ascorbic acid) Content in Fruit Extracts by Redox Titration

To determine the vitamin C content in fruit extracts, 20 mL of aqueous fruit extract was mixed with 150 mL of distilled water and 1 mL of starch in a conical flask. The mixture was then titrated with a 0.005 M iodine solution. The endpoint of the titration was indicated by the appearance of a dark blue-black color. Each measurement was performed in triplicate. The vitamin C content was reported in mg/100 g (Devolli *et al.,* 2021). Statistical analysis was

conducted using one-way ANOVA and Tukey's pairwise comparison, with data processed using SPSS software.

2.11. Determination of Nutrient Content in Fruits

Proximate analysis was conducted on 250 g of fresh fruit samples from each species to assess their fat, protein, carbohydrate, moisture, ash, and energy content (Industrial Technology Institute, Sri Lanka).

3. FINDINGS

3.1. Qualitative Phytochemical Screening of the Fruit Extracts

The study analyzed 10 fruit extracts and found that all contained polyphenols, compounds known for their antioxidant properties and commonly present in fruits. Flavonoids and saponins, which are also beneficial phytochemicals, were detected in all the fruits except *B. motleyana* (Table 1). Tannins, another type of polyphenol, were not detected in both *B. motleyana* and *A. ghaesembilla*. Alkaloids were found only in the water and/or acetone extracts of *A. alexiteria*, *C. cauliflora*, *P. pussilla*, and *E. angustifolius*. These results offer valuable insights into the phytochemical profiles of the fruits studied, highlighting their potential nutritional and health benefits.

Table 1. Qualitative phytochemicals screening of fresh fruit extracts in methanol (M), water (W) and acetone (A).

Fruit species	Extraction	Polyphenol: $FeCl_3$ Test	Flavonoids	Saponins	Tannins	Alkaloids
S. caryophyllatum	M	✓	✓	✓	✓	×
	W	✓	✓	✓	✓	×
	A	✓	✓	✓	✓	×
M. paniculata	M	×	✓	✓	×	×
	W	×	✓	✓	×	×
	A	✓	✓	✓	✓	×
A. ghaesembilla	M	✓	✓	✓	×	×
	W	×	✓	✓	×	×
	A	✓	✓	✓	×	×
A. alexiteria	M	✓	✓	✓	✓	×
	W	✓	✓	✓	✓	✓
	A	✓	✓	✓	✓	✓
B. motleyana	M	✓	×	×	×	×
	W	×	×	×	×	×
	A	×	×	×	×	×
C. cauliflora	M	✓	✓	✓	×	×
	W	×	✓	✓	✓	✓
	A	✓	✓	✓	✓	×
P. pussilla	M	✓	✓	×	✓	×
	W	×	✓	✓	✓	✓
	A	✓	✓	✓	✓	×
P. guineense	M	✓	✓	×	✓	×
	W	✓	✓	✓	×	×
	A	✓	✓	✓	✓	×
Z. oenoplia	M	✓	✓	✓	✓	×
	W	✓	✓	×	✓	×
	A	✓	✓	✓	✓	×
E. angustifolius	M	✓	✓	×	✓	×
	W	✓	✓	✓	✓	×
	A	✓	✓	✓	✓	✓

3.2. Total Phenolic Content of Fresh Fruit Extracts

The Folin-Ciocalteu assay revealed the presence of phenolics in all 10 fruit species (Table 2). The phenolic content in methanol extracts had levels ranging from 1.18 to 7.15 mg GAE/g FW, aqueous extracts ranged from 1.69 to 40.20 mg GAE/g FW, and acetone extracts ranged from 1.16 to 169.22 mg GAE/g FW. The highest phenolic content in methanol extracts was found in *S. caryophyllatum* (7.15 mg GAE/g FW), aqueous extracts in *Z. oenoplia* (40.20 mg GAE/g FW), and acetone extracts in *P. pussilla* (169.22 mg GAE/g FW). Notably, *P. pussilla* had the highest total phenolic content in acetone extracts (169.22 mg GAE/g FW), followed by *Z. oenoplia* in water extracts (40.20 mg GAE/g FW). Additionally, acetone extracts of *M. paniculata* (25.75 mg GAE/g FW), *S. caryophyllatum* (21.16 mg GAE/g FW), and *C. cauliflora* (21.36 mg GAE/g FW) also exhibited relatively high phenolic contents. The significant variation in total phenolic content among the fruit species, depending on the solvent used, underscores the considerable impact of solvent type on the measurement.

Table 2. Total phenolic content of fresh fruit extracts (mg GAE/g FW).

Fruit species	Total phenolic content of fresh fruit extracts (mg GAE/g FW)		
	Methanol extract	Water extract	Acetone extract
S. caryophyllatum	7.15 ± 0.65 ^{e}	6.50 ± 0.50 ^{d}	21.16 ± 2.0 ^{bc}
M. paniculata	2.79 ± 0.24 ^{bc}	2.74 ± 0.04 ^{abc}	25.75 ± 1.9 ^{c}
A. ghaesembilla	3.47 ± 1.18 ^{cd}	3.39 ± 0.40 ^{abc}	8.08 ± 0.10 ^{abc}
A. alexiteria	4.38 ± 0.49 ^{d}	2.15 ± 0.26 ^{ab}	8.95 ± 0.40 ^{abc}
B. motleyana	4.47 ± 0.29 ^{d}	4.26 ± 0.41 ^{bc}	1.16 ± 0.30 ^{a}
C. cauliflora	2.82 ± 0.21 ^{bc}	1.69 ± 0.26 ^{a}	21.36 ± 0.80 ^{bc}
P. pussilla	1.53 ± 0.11 ^{ab}	1.69 ± 0.26 ^{a}	169.22 ± 20.4 ^{d}
P. guineense	1.92 ± 0.14 ^{ab}	4.77 ± 0.66 ^{cd}	8.32 ± 0.60 ^{abc}
Z. oenoplia	1.18 ± 0.06 ^{a}	40.20 ± 2.01 ^{e}	9.85 ± 0.70 ^{abc}
E. angustifolius	1.71 ± 0.13 ^{ab}	4.71 ± 0.45 ^{cd}	6.35 ± 1.4 ^{ab}

3.3. Total Flavonoid Content of Fresh Fruit Extracts

Flavonoids were detected in all 10 fruit samples tested (Table 3). The total flavonoid content varied across different solvents: methanol extracts ranged from 0.01 to 0.45 mg QE/g FW, aqueous extracts from 0.11 to 0.97 mg QE/g FW, and acetone extracts from 0.01 to 0.54 mg QE/g FW. *E. angustifolius* showed the highest flavonoid content in methanol (0.45 mg QE/g FW), *P. pussilla* in water (0.97 mg QE/g FW), and *A. ghaesembilla* in acetone (0.54 mg QE/g FW). The highest total flavonoid content overall was found in the water extract of *P. pussilla* (0.97 mg QE/g FW). These results also highlight the significant impact of solvent type on the flavonoid content in various fruit species.

Table 3. Total flavonoid content of fresh fruit extracts (mg QE/g FW).

Fruit species	Total flavonoid content of fresh fruit extracts (mg QE/g FW)		
	Methanol extract	Water extract	Acetone extract
S. caryophyllatum	0.35 ± 0.02 ^{f}	0.28 ± 0.04 ^{c}	0.47 ± 0.05 ^{ef}
M. paniculata	0.18 ± 0.00 ^{cde}	0.33 ± 0.04 ^{c}	0.39 ± 0.03 ^{e}
A. ghaesembilla	0.11 ± 0.01 ^{bcd}	0.23 ± 0.01 ^{abc}	0.54 ± 0.02 ^{f}
A. alexiteria	0.11 ± 0.01 ^{bc}	0.14 ± 0.01 ^{ab}	0.15 ± 0.04 ^{bc}
B. motleyana	0.01 ± 0.01 ^{a}	0.11 ± 0.07 ^{a}	0.01 ± 0.01 ^{a}
C. cauliflora	0.05 ± 0.02 ^{ab}	0.13 ± 0.05 ^{a}	0.08 ± 0.01 ^{ab}
P. pussilla	0.18 ± 0.00 ^{cde}	0.97 ± 0.11 ^{d}	0.30 ± 0.03 ^{d}
P. guineense	0.19 ± 0.06 ^{de}	0.22 ± 0.02 ^{abc}	0.24 ± 0.02 ^{cd}
Z. oenoplia	0.24 ± 0.01 ^{e}	0.26 ± 0.02 ^{bc}	0.14 ± 0.04 ^{b}
E. angustifolius	0.45 ± 0.05 ^{g}	0.13 ± 0.01 ^{a}	0.26 ± 0.02 ^{d}

3.4. Antioxidant Activity of Fresh Fruit Extracts - DPPH Assay

The DPPH free radical scavenging activity of 10 fruit extracts was evaluated using methanol, acetone, and water as solvents. Methanolic extracts exhibited DPPH scavenging activity ranging from 2.41 to 14.18 TE µmol/g FW. Aqueous extracts showed values between 0.53 and 9.57 TE µmol/g FW, while acetone extracts ranged from 1.52 to 280.31 TE µmol/g FW (Table 4). The highest scavenging activity was found in the methanolic extract of *S. caryophyllatum* (14.18 TE µmol/g FW), the aqueous extract of *Z. oenoplia* (9.57 TE µmol/g FW), and the acetone extract of *P. pussilla* (280.31 TE µmol/g FW). These results indicate that the choice of solvent significantly influences the DPPH scavenging activity of fruit extracts, with certain fruits showing particularly strong antioxidant properties depending on the solvent used. Notably, acetone extracts of *P. pussilla* demonstrated the highest antioxidant activity.

Table 4. Antioxidant activity of fresh fruit extracts; DPPH free radical scavenging assay (TE µ mol/g FW).

Fruit species	DPPH free radical scavenging assay (TE µ mol/g FW)		
	Methanol extract	Water extract	Acetone extract
S. caryophyllatum	14.18 ± 5.01 ^{c}	3.03 ± 0.90 ^{b}	42.96 ± 2.17 ^{a}
M. paniculata	12.15 ± 0.61 ^{c}	0.72 ± 0.10 ^{a}	33.72 ± 0.42 ^{a}
A. ghaesembilla	5.07 ± 1.56 ^{a}	1.17 ± 0.80 ^{a}	21.56 ± 4.45 ^{a}
A. alexiteria	5.42 ± 1.37 ^{ab}	0.92 ± 0.30 ^{a}	14.06 ± 1.10 ^{a}
B. motleyana	3.21 ± 1.48 ^{a}	0.69 ± 0.40 ^{a}	1.52 ± 1.16 ^{a}
C. cauliflora	10.48 ± 0.69 ^{bc}	0.72 ± 0.50 ^{a}	29.92 ± 1.58 ^{a}
P. pussilla	2.41 ± 0.27 ^{a}	8.63 ± 0.20 ^{c}	280.31 ± 159.74 ^{b}
P. guineense	13.00 ± 0.78 ^{c}	1.93 ± 0.40 ^{c}	3.28 ± 2.51 ^{a}
Z. oenoplia	6.15 ± 0.64 ^{ab}	9.57 ± 0.80 ^{ab}	26.93 ± 5.63 ^{a}
E. angustifolius	5.18 ± 0.72 ^{ab}	0.53 ± 0.40 ^{ab}	9.63 ± 1.97 ^{a}

3.5. Antioxidant Activity of Fresh Fruit Extracts - FRAP Assay

The ferric-reducing antioxidant power (FRAP) of 10 fruit extracts was assessed using methanol, aqueous, and acetone solvents. The FRAP values varied as follows: methanol extracts ranged from 1.29 to 16.28 $FeSO_4$ µmol/g FW, aqueous extracts from 5.92 to 294.85 $FeSO_4$ µmol/g FW, and acetone extracts from 19.83 to 138.75 $FeSO_4$ µmol/g FW (Table 5). The highest FRAP values were recorded in the methanol extract of *M. paniculata* (16.28 $FeSO_4$ µmol/g FW), the aqueous extract of *Z. oenoplia* (294.85 $FeSO_4$ µmol/g FW), and the acetone extract of *P. pussilla* (138.75 $FeSO_4$ µmol/g FW). Notably, acetone extracts of most fruits (excluding *P. pussilla* and *Z. oenoplia*) demonstrated higher antioxidant capacity compared to their water and methanol counterparts.

Table 5. Antioxidant activity of fresh fruit extracts; FRAP assay ($FeSO_4$ µmol/g FW).

Fruit species	FRAP assay ($FeSO_4$ µmol/g FW)		
	Methanol extract	Water extract	Acetone extract
S. caryophyllatum	10.66 ± 3.62 ^{bc}	15.11 ± 9.37 ^{a}	84.22 ± 7.51 ^{d}
M. paniculata	16.28 ± 0.97 ^{c}	5.92 ± 5.05 ^{a}	88.98 ± 12.31 ^{d}
A. ghaesembilla	1.56 ± 1.30 ^{a}	17.14 ± 0.24 ^{a}	26.21 ± 9.68 ^{ab}
A. alexiteria	1.29 ± 1.04 ^{a}	10.10 ± 2.36 ^{a}	42.88 ± 5.43 ^{bc}
B. motleyana	1.66 ± 0.40 ^{a}	4.74 ± 0.12 ^{a}	50.58 ± 0.62 ^{c}
C. cauliflora	8.11 ± 4.62 ^{ab}	4.58 ± 0.27 ^{a}	51.81 ± 11.95 ^{c}
P. pussilla	6.12 ± 0.67 ^{ab}	245.34 ± 40.81 ^{b}	138.75 ± 6.85 ^{e}
P. guineense	8.03 ± 5.89 ^{ab}	30.44 ± 7.52 ^{a}	45.49 ± 5.19 ^{bc}
Z. oenoplia	4.60 ± 2.31 ^{ab}	294.85 ± 42.67 ^{b}	30.05 ± 7.31 ^{abc}
E. angustifolius	9.86 ± 0.87 ^{bc}	7.24 ± 0.62 ^{a}	19.83 ± 4.45 ^{a}

3.6. Antioxidant Activity of Fresh Fruit Extracts - ABTS Assay

The ABTS radical scavenging activity of 10 fruit extracts was evaluated using methanol, aqueous, and acetone solvents. The activity ranged from 1.44 to 30.39 TE µmol/g FW in methanol extracts, 1.94 to 241.16 TE µmol/g FW in aqueous extracts, and 5.25 to 60.46 TE µmol/g FW in acetone extracts. *S. caryophyllatum*, *P. guineense*, and *C. cauliflora* exhibited the highest ABTS scavenging activity in methanol extracts, while *P. pussilla* showed the highest antioxidant power in aqueous extracts. For acetone extracts, the highest ABTS activity was found in *S. caryophyllatum*, *M. paniculata*, *A. alexiteria*, *C. cauliflora*, and *Z. oenoplia*. The highest antioxidant power, 241.16 TE µmol/g FW, was recorded in the aqueous extract of *P. pussilla* (Table 6).

Table 6. Antioxidant activity of fresh fruit extracts; ABTS assay (TE µ mol/g FW).

Fruit species	ABTS assay (TE µ mol/g FW)		
	Methanol extract	Water extract	Acetone extract
S. caryophyllatum	30.39 ± 3.30 ^{d}	5.85 ± 1.73 ^{a}	43.17 ± 4.51 ^{cd}
M. paniculata	12.16 ± 1.32 ^{b}	3.34 ± 0.41 ^{a}	47.81 ± 13.23 ^{d}
A. ghaesembilla	13.21 ± 4.26 ^{b}	1.94 ± 0.81 ^{a}	10.46 ± 3.47 ^{a}
A. alexiteria	12.13 ± 1.13 ^{b}	3.65 ± 0.34 ^{a}	41.58 ± 7.05 ^{bcd}
B. motleyana	2.04 ± 1.15 ^{a}	3.87 ± 2.20 ^{a}	15.50 ± 6.90 ^{ab}
C. cauliflora	19.20 ± 3.57 ^{bc}	4.12 ± 0.47 ^{a}	60.46 ± 3.67 ^{d}
P. pussilla	1.44 ± 0.85 ^{a}	241.16 ± 166.63 ^{b}	11.53 ± 7.77 ^{a}
P. guineense	24.75 ± 3.17 ^{cd}	5.33 ± 1.41 ^{a}	18.48 ± 18.88 ^{abc}
Z. oenoplia	14.67 ± 5.39 ^{b}	16.91 ± 0.56 ^{a}	38.24 ± 8.16 ^{bcd}
E. angustifolius	15.81 ± 3.18 ^{bc}	3.49 ± 1.41 ^{a}	5.25 ± 6.48 ^{a}

3.7. Ascorbic Acid Content in Fruit Extracts

The ascorbic acid content in the fruit extracts ranged from 21.72 to 489.03 mg/100 g FW. The highest ascorbic acid levels were observed in *P. pussilla* (489.03 mg/100 g FW), followed by *S. caryophyllatum* (209.58 mg/100 g FW) and *A. alexiteria* (160.27 mg/100 g FW) (Table 7). The lowest ascorbic acid content was found in *E. angustifolius* (21.72 mg/100 g FW), with *B. motleyana* having slightly higher levels at 31.11 mg/100 g FW.

Table 7. The ascorbic acid content of aqueous fresh fruit extracts.

Fruit species	Ascorbic acid content (mg/100 g)
S. caryophyllatum	209.58 ± 7.04 ^{b}
M. paniculata	90.41 ± 4.43 ^{e}
A. ghaesembilla	46.97 ± 2.69 ^{f}
A. alexiteria	160.27 ± 1.79 ^{c}
B. motleyana	31.11 ± 2.69 ^{h}
C. cauliflora	110.96 ± 4.66 ^{d}
P. pussilla	489.03 ± 2.03 ^{a}
P. guineense	104.50 ± 3.67 ^{d}
Z. oenoplia	72.80 ± 4.07 ^{d}
E. angustifolius	21.72 ± 3.67 ^{h}

3.8. Nutrient Content in Fruits

The proximate analysis of selected underutilized fruits assessed their fat, protein, carbohydrate, moisture, ash content, and energy (kcal/100 g) (Table 8). *A. alexiteria* had the highest fat content at 1.1%, while *B. motleyana* had the lowest at 0.1%. The highest protein content was found in *P. pussilla* (3.4%), and the lowest in *A. alexiteria* (0.4%). Carbohydrate percentages ranged from 9.5% in *C. cauliflora* to 38.7% in *P. guineense*, with the highest energy content in

P. guineense and the lowest in *C. cauliflora*. *C. cauliflora* also had the highest moisture content at 88.9%, while *P. guineense* had the highest ash content at 1.5%.

Table 8. Proximate analysis of the selected underutilized fruits.

Fruit species	Fat percent by mass	Protein percent by mass	Carbohydrates percent by mass	Energy kcal/100 g	Moisture percent by mass	Ash percent by mass
S. caryophyllatum	0.3	1.1	36.2	152	61.6	0.8
M. paniculata	0.5	2.5	19.1	91	76.9	1
A. ghaesembilla	0.8	1.4	18.7	88	78.1	1
A. alexiteria	1.1	0.4	18.3	85	79	1.2
B. motleyana	0.1	1.5	14.1	63	83.3	1.0
C. cauliflora	0.4	0.8	9.5	45	88.9	0.4
P. pussilla	0.4	3.4	35.6	160	59.8	0.8
P. guineense	0.7	2.7	38.7	172	56.4	1.5
Z. oenoplia	0.5	2.4	36.7	161	59.0	1.4
E. angustifolius	0.4	2.8	25.1	115	70.7	1

4. DISCUSSION

Preliminary qualitative phytochemical screening of fruits can provide valuable insights into the quantitative estimation of their chemical constituents, helping to assess their potential as sources of pharmacologically active compounds. In this study, preliminary phytochemical screening identified the presence of polyphenols, tannins, flavonoids, saponins, and alkaloids in varying concentrations in several underutilized fruits. Polyphenols, known for their free radical scavenging abilities, play a significant role in mitigating oxidative stress and protecting against chronic diseases such as coronary heart disease, cancer, and diabetes (Asami *et al.*, 2003). Tannins not only exhibit antioxidant properties but also possess antimicrobial, antiviral, and anti-inflammatory effects (Akiyama *et al.*, 2001). Saponins, prevalent in many plants, are associated with antioxidant, anticancer, and immune-boosting activities, indicating their potential in disease treatment (Güçlü-Üstündağ & Mazza, 2007). Alkaloids contribute antibacterial, anti-inflammatory, and analgesic benefits (Dewi & Purwayantie, 2019). The choice of solvent for extracting plant secondary metabolites influences extraction efficiency, as compounds vary in polarity and solubility (Singh & Kumar, 2017). Therefore, choosing the right solvent for extracting bioactive compounds from fruit samples is a challenging task. This study utilized methanol, acetone, and water as solvents for extraction.

Our research demonstrated that the total phenolic content (TPC) in the 10 underutilized native fruit species was significantly higher compared to commonly available imported fruits in Sri Lanka. For instance, the TPC values for red apple, red grape, and orange were 73.96, 80.28, and 77.23 mg GAE/100 g FW, respectively (Fu *et al.*, 2011). In contrast, the TPC of Australian Cavendish banana pulp was much lower at 0.43 mg GAE/g FW (Bashmil *et al.*, 2021). Mango, often referred to as the "king of fruits," had a TPC range of 1.39 - 0.32 mg GAE/g FW (Liu, 2013), while date fruit cultivars showed higher TPC values ranging from 100 - 350 mg GAE/g FW (Allaith, 2019). Notably, *P. pussilla*, a wild date species, exhibited the highest TPC content of 169 mg GAE/g FW in acetone fruit extracts. The TPC values for *S. caryophyllatum* ranged from 1.72 - 8.92 mg GAE/g, consistent with previous reports by Wathsara *et al.*, (2020). The yield of total phenols can vary based on extraction methods and solvent choice. Factors such as

season, genetics, agronomic conditions, maturation stages, temperature, and rainfall also affect TPC in plant tissues (Goli *et al.,* 2005).

Total flavonoid content (TFC) in the 10 fruit extracts was measured using the aluminum chloride method, which assesses the reaction between aluminum chloride and flavonoid carbonyl groups to form stable complexes. Flavonoids, including flavones, flavanols, and condensed tannins, offer potential health benefits and protection against diseases associated with oxidative stress (Bahramikia *et al.,* 2009). TFC values ranged from 0.01 - 1.00 mg QE/g FW in fresh fruit extracts studied in the present research. Studies from Burkina Faso found flavonoid contents in banana varieties ranging from 1.7 to 116.05 mg QE/100 g for methanolic extracts and from 5.3 to 155.9 mg QE/100 g for acetone extracts, with acetone proving more effective (Lamien-Meda *et al.,* 2008). Similarly, this study found that acetone extracts of the studied fruits were more effective at extracting flavonoids than methanol extracts. This is consistent with findings by Lamien-Meda *et al.,* (2008). Antolovich *et al.* (2000) indicated low solubility of flavonoids in aqueous media and hence lower TFC in water extracts.

Quantitative measures of antioxidant capacity involve various assays, such as DPPH, FRAP, and ABTS, to obtain a comprehensive understanding of antioxidant properties (Noipa *et al.,* 2011; Moharram & Youssef, 2014). The DPPH assay evaluates the ability of antioxidants to donate hydrogen atoms to the stable free radical 2,2-diphenyl-1-picrylhydrazyl (Sánchez-Moreno, 2002). The antioxidant activity of the 10 fruits ranged from 0.69 - 280.31 TE µmol/g FW across the three solvents, with acetone extracts generally showing higher radical scavenging capacity than methanol and water extracts. Similar research on the antioxidant activity of fruits using the DPPH assay has been carried out in Turkey. Methanol extracts from five blackberry cultivars demonstrated higher antioxidant values compared to aqueous extracts (Sariburun *et al.,* 2010). In Burkina Faso, acetone extracts from 15 wild edible fruits showed high DPPH scavenging activities (319.63 - 8709.5 mg AEAC/100 g of FW for methanol extracts and 499.48 -10729.41 mg AEAC/100 g of FW for acetone extracts) (Lamien-Meda *et al.,,* 2008). In Ecuador, the DPPH radical scavenging capacities of guava, strawberry, passion fruit, and mango were 30, 11, 0.5, and 3.1 TE µmol/g FW, respectively (Vasco *et al.,,* 2008). Almost all the 10 wild fruit species examined in this study exhibited higher antioxidant values than these commonly consumed fruits, indicating a greater antioxidant potential.

The FRAP assay directly measures the reducing potential of antioxidant compounds by reacting them with a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex, producing a colored ferrous tripyridyltriazine (Fe^{2+}-TPTZ) (Benzie & Strain, 1996). FRAP values of the fresh fruit extracts studied in this research ranged from 1.29 - 294 $FeSO_4$ µmol/g FW, with acetone extracts generally showing higher values. It was observed that acetone extracts exhibited higher FRAP values in most of the tested fruits, consistent with the findings of Lamien-Meda *et al*. (2008). Proteggente *et al*. (2002) investigated the antioxidant activity of regularly consumed fruits; apples, bananas, and grapes, using the FRAP assay. The study reported the following values for methanolic (50%) extracts: 3.94, 1.64, and 8.29 $FeSO_4$ µmol/g FW, respectively. Interestingly, the FRAP values for methanolic extracts of most of the fruits studied in the present research were higher than those reported in the above findings. Silva & Sirasa (2018) evaluated the antioxidant activity of various fruit cultivars in Sri Lanka and found that methanolic (80%) extracts of underutilized fruits such as *Phyllanthus emblica*, *Averrhoa carambola*, and *Annona squamosa* exhibited high FRAP values, with readings of 1022.05, 46.75, and 41.50 $FeSO_4$ µmol/g FW, respectively.

The ABTS assay measures the antioxidant capacity of hydrogen-donating antioxidants (Re *et al.,* 1999). In this study, ABTS antioxidant capacity ranged from 2.04 - 241.16 TE µmol/g FW, with acetone extracts again showing higher values compared to aqueous and methanol extracts, similar to the FRAP assay. Sariburun *et al*. (2010) reported the ABTS activity of water and methanolic extracts from raspberry cultivars in Turkey, with values ranging from 64.36 to 83.00 TE µmol/g FW for water extracts and from 72.92 to 117.07 TE µmol/g FW for methanolic

extracts. Fu *et al*. (2011) reported ABTS antioxidant activity values for various fruits, including apples (Red Delicious), avocados, bananas, oranges (South Africa), and grapes (USA), with values of 4.98, 1.16, 3.44, 4.90, and 1.23 TE µmol/g FW, respectively. These values were lower than the ABTS antioxidant activity observed in most of the wild indigenous fruits studied in the current research.

The antioxidant activity of polar solvent extracts (water, methanol, and acetone) was relatively higher than non-polar solvents. The type and polarity of the extracting solvent can have a substantial effect on the antioxidant activity of a sample. Some of the tested fruits exhibited high Trolox equivalent values, indicating strong antioxidant activity. However, their total phenolic content, measured as GAE, was relatively low. This suggests that these fruits may contain other powerful phenolic antioxidants that enhance their antioxidant activity, despite the modest total phenolic content.

Ascorbic acid, commonly known as vitamin C, is recognized for its antioxidant properties and its role in preventing oxidative damage in the body. A deficiency in ascorbic acid can lead to health issues. According to Benzie (2003), the recommended daily intake of vitamin C is 75 mg for women and 90 mg for men. The 10 fruit species examined in this study contained ascorbic acid in varying amounts, ranging from 21.72 to 489.03 mg/100 g FW. These fruits show potential as sources of natural ascorbic acid. Their ascorbic acid content is higher compared to commonly available fruits in Sri Lanka, such as *Mangifera indica* (30.8 mg/100 g FW), *Ananas comosus* (15.1 mg/100 g FW), *Musa paradisiaca* AAB "Mysore" (2.3 mg/100 g FW), *Persea americana* (5.0 mg/100 g FW), and *Nephelium lappaceum* (18.5 mg/100 g FW) (Abeysuriya *et al.*,, 2020). Wild edible fruits from the Indian Himalayan region, including *Phyllanthus emblica* (3315 mg/100 g FW), *Morus alba* (2953 mg/100 g FW), *Ficus palmata* (727 mg/100 g FW), and *Terminalia chebula* (626 mg/100 g FW), have demonstrated high ascorbic acid content (Bhatt *et al.*, 2017).

The current study demonstrates that the indigenous fruit species *P. pussilla*, *A. ghaesembilla*, *A. alexiteria*, and *S. caryophyllatum* possess significant antioxidant properties, making them potential candidates for use in natural food colors, cosmetics, and pharmaceuticals. The proximate analysis determined the nutritional composition, including moisture, ash, protein, carbohydrates, energy, and fat percentages of 10 underutilized fruit species. Silva *et al*. (2015) investigated six underutilized Arecaceous fruits in Brazil (*Acrocomia intumescens*, *Pinanga kuhlii*, *Ptychosperma macarthuri*, *Syagrus cearensis*, *Syagrus coronata*, and *Veitchia merrillii*) and found that fruit pulp moisture levels ranged from 60% to 75%, except for *P. kuhlii* (22.9%), with carbohydrate contents between 1.5% and 20.6%. Another study by De Souza *et al*. (2014) on berry fruits reported moisture content ranging from 86.43% (cherry) to 92.68% (strawberry), ash content from 0.08% (blueberry) to 0.42% (cherry), fat content from 0.19% (blueberry) to 0.42% (blackberry), and protein content from 0.48% (blueberry) to 1.27% (blackberry). In contrast, the fruits tested in the present study exhibited lower moisture content and higher levels of ash, carbohydrates, and protein. This low moisture content may contribute to extended storage life, making these fruits suitable for longer-term use.

Research on the antioxidant properties of underutilized fruits in Sri Lanka is limited, highlighting a gap in scientific data on the bioactivity of secondary metabolites (Abeysuriya *et al.*,, 2020). This study represents a significant advancement in this field, by examining the total phenolic content, flavonoid content, and antioxidant activities of several underutilized fruits, including *P. pussilla*, *A. ghaesembilla*, *A. alexiteria*, and *Z. oenoplia*, using three different solvent extracts. The findings confirm that these fruits are valuable for their nutritional and health-promoting properties. The fruit species investigated have substantial commercial potential in Sri Lanka and could significantly contribute to food and nutritional security, particularly in urban and rural communities, while also promoting local cultivation and consumption. Raising awareness of the health benefits of these underutilized fruits could foster their cultivation, commercialization, and consumption, providing a strategic approach to

address the rising issue of non-communicable diseases in Sri Lanka. The findings of this study emphasize the need for further investigation to identify the phytochemicals present in these fruits through HPLC analysis.

5. CONCLUSION

The fruits of *P. pussilla* and *S. caryophyllatum* exhibited the highest levels of total phenolic content, flavonoid content, and ascorbic acid content. Additionally, *P. pussilla*, *A. ghaesembilla*, *A. alexiteria*, and *S. caryophyllatum* demonstrated significant antioxidant properties, suggesting their potential for various industrial applications. The total phenolic and flavonoid contents, as well as antioxidant activities, varied with the type of solvent used for extraction. Specifically, a 60% acetone aqueous solution proved to be the most effective solvent for extracting total phenolics, flavonoids, and antioxidants from the selected fruits. The fruits analyzed in this study had low moisture content, along with high ash, carbohydrate, and protein levels, with the low moisture content contributing to an extended shelf life.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Indi Vishaka Somasiri: Investigation, Data analysis, Writing original draft of the manuscript. **Harshini Herath**: Conceptualization, Research supervision, Writing, reviewing and editing the manuscript. **Sena Ratnayake**: Conceptualization, Research supervision, Reviewing and editing the manuscript. **Priyanganie Senanayake**: Conceptualization, Research supervision, Reviewing and editing the manuscript.

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