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Functional Properties and Physicochemical Composition of Different Leaf Positions of Soft and Firm Flesh Trees of *Artocarpus heterophyllus* Lam. (Moraceae)

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Abstract The present study was undertaken to determine the functional properties and physicochemical composition of different leaf positions (Leaf bud, first leaf, second leaf, third leaf and fourth leaf) of soft (*Wela*) and firm (*Waraka*) flesh trees of jackfruit. Physicochemical composition, total phenolic content (TPC), total flavonoid content (TFC.) and total antioxidant capacity (TAC) were determined using the official AOAC method, Folin-Ciocalteu method, colourimetric method and Ferric Reducing Antioxidant Power (FRAP) assay respectively. Among the leaf bud and leaf blades, significantly highest TPC, TFC, and TAC. were observed in the second leaf blade of the soft flesh type, whereas the significantly highest TPC, TFC. and TAC. were recorded in the first leaf blade of the firm flesh type. Among the leaf petioles, significantly highest TPC, TFC. and TAC were observed in the fourth petiole of the soft flesh type, whereas, in the firm flesh type, the second petiole had significantly highest TPC, TFC. and TAC. However, the soft flesh type had more bioactive compounds and antioxidant capacity compared to the firm flesh type. Higher crude protein content was observed in leaf buds, whereas higher moisture and ash content were observed in leaf petioles in both types. According to the results, it could be concluded that all the tested leaf positions of both *A. heterophyllous* types contained significant amounts of TPC, TFC, and TAC, which can be effectively used for traditional or folk systems of medicine and pharmaceutical industries.

Keywords: *Artocarpus heterophyllus, Firm and soft flesh, Functional properties, Leaf positions, Physicochemical composition*

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1. Introduction

Artocarpus heterophyllus Lam. is a multi-purpose, medium-sized, evergreen, tropical perennial tree belonging to the family Moraceae [1]. Jackfruit is native to the Western Ghats of India and distributed in Asia, Africa and some regions of South America [1]. *Artocarpus heterophyllus* is commonly known in English as Jackfruit, *Kos* in Sinhala and *Palaa* in Tamil [2]. According to the softness or firmness of the flesh/aril of the fruitlets at the ripening stage, there are two major types of *A. heterophyllus* known as soft flesh type and firm flesh type. The soft flesh type is locally called *wela*, and the firm flesh type is *waraka* [3]. *A.heterophyllus* has broad, obovate or elliptic, glossy dark green leaves, which are inserted alternately on horizontal branches but tend to spiral on ascending branches. Leaves are 10-15 cm in length and have a pinnate venation pattern with 5-8 pairs of veins. The juvenile leaves are deeply lobed. The leaf petiole is dark green and approximately 3.5 cm long [4]. The leaves of jackfruit contain therapeutically important phytochemicals such as sapogenin, cycloaratenone, cycloartenol, β-sitosterols, flavanones, polyphenols and tannins [5]. Due to the presence of an array of phytochemicals and biologically active molecules, jackfruit leaves have been used for the treatment of skin diseases, fever, boils, wounds and vitiated conditions of *Pitta* and *Vata*. Also, the ash of the leaves is useful in healing ulcers [5]; apart from that, the glucose tolerance of healthy and non-insulin-dependent diabetic patients can be improved significantly by using leaf decoctions or hot water extract of leaves [1,3]. In addition, the leaves of jackfruits are also help to increase lactation in women and animals and are used to treat anaemia, asthma, dermatitis, diarrhoea and cough [6]**.** The shoots of jackfruit also have some nematicidal activity against various nematodes, including *Rotylenchulus reniformis, Tylenchorhynchus brassicae*, *Tylenchus filifofmis* and *Meloidogyne incognita* [2]. Traditionally, the decoction of the mature leaf petioles of jackfruit is also used in various properties such as anti-inflammatory and antimicrobial agents due to the presence of phenols, flavonoids and alkaloids [7].

Despite such vast usefulness and health benefits, unfortunately, jackfruit remains an underutilized crop. It is also not classified as a commercial crop and is rarely grown on regular plantations [2]. Though jackfruit leaves have been widely studied, and the maturity level of jackfruit leaves and their functional properties of leaves at different leaf maturity stages and petiole at different leaf positions of soft flesh type and firm flesh type still needs to be improved. Therefore, the present study was carried out to determine the functional properties and physicochemical compositions of different leaf positions of soft and firm flesh trees of *A. heterophyllus*.

2. Materials and Methods

2.1. Location

The study was carried out in the laboratory of the Department of Plantation Management, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP), from November 2022 to March 2023.

2.2. Sample Collection

Healthy fresh leaves from different leaf positions (Leaf bud, first, second, third and fourth leaves) of soft (*Wela*) and firm flesh (*Waraka*) trees of *A. heterophyllus* were collected from previously authenticated plants available at university premises. Homogeneous representative samples were collected and mixed to make composite samples.

2.3. Sample Preparation

After collecting the leaf samples, leaf blades and leaf petioles were separated and thoroughly washed under running water. Washed samples were cut into small pieces and air-dried for three days at room temperature (28 ± 2 ˚C). All air-dried samples were powdered using a coffee grinder and sieved with a 0.25 mm mesh. Powdered samples were stored at -20 ˚C until methanolic extraction.

2.4. Extraction of Phytochemicals

The powdered sample (0.1 g) was mixed with 5 mL of 80% methanol and vortexed for 15 min. Then it was kept in a water bath at 60 ˚C for 40 min. Then the vortex procedure was continued in 10 min intervals and centrifuged at 4,000 rpm for five minutes to remove the solid fraction; the supernatant was decanted into a 15 mL falcon tube, and the remaining was re-extracted with 80% methanol. Supernatant stored at -20 ˚C until analysis. All the samples were extracted and analyzed in 3 replicates.

2.5. Qualitative Screening of Phytochemicals

Methanolic extracts of samples were screened for flavonoids, saponins, steroid glycosides and tannins according to the method described by Farnsworth [8] with slight modifications.

2.6. Determination of Functional Properties

The total phenolic content (TPC) of all samples was determined using a modified Folin-Ciocalteu method [9]. TPC was determined and expressed as mg of gallic acid equivalents (GAE) per g of DW. Total flavonoid content (TFC) was calculated by using a colourimetric method [10] with slight modifications. The values were calculated using the standard rutin curve and expressed as mg of rutin equivalents (RE) per g DW.

Total antioxidant capacity (TAC) was determined using the Ferric Reducing Antioxidant Power (FRAP) assay described by Benzie and Strain [11] with slight modifications. TAC was determined using the standard Trolox curve and expressed as mg of Trolox equivalents (TE) per g of DW.

2.7. Determination of Physicochemicals

Under the determination of physicochemical properties, moisture, ash and crude protein contents were determined by using the Standard protocol published in AOAC [12].

2.8. Statistical Analysis

Statistical significance of mean values was performed by General Linear Model (GLM) of ANOVA followed by Tukey's Multiple Range Test using SAS statistical software (version 9.4). The P values, which are less than 0.05 were considered statistically significant.

3. Results and Discussion

3.1. Phytochemical Screening

Results of preliminary phytochemical screening are demonstrated in Table 1. The results revealed that all tested phytochemicals, namely flavonoids, saponins, steroid glycosides and tannins, are present in leaf buds, leaf blades and leaf petioles of all tested leaf positions of both *A. heterophyllus* types. The results obtained from this study agree with the findings of Okonkwo *et al*. [2], who reported that the presence of alkaloids, flavonoids, glycosides, saponins, tannins and steroids in ethanol leaf extract and also the findings of Thapa *et al*. [13] who reported that presence of tannins and saponins in aqueous methanolic extract of leaves of *A. heterophyllus*. The steroid is responsible for reducing cholesterol, and also saponin can lower blood cholesterol and inhibit cancer cell growth. Moreover, it is a well-known fact that medicinal properties of jackfruit leaves are due to the presence of phytochemicals or secondary metabolites in leaves [13].

Table 1. Qualitative phytochemical parameters of different leaf positions of soft and firm flesh trees of *Artocarpus heterophyllus*

Plant material	F	S	St	T
Leaf bud (SF)	+	+	+	+
1st Leaf blade (SF)	+	+	+	+
2nd Leaf blade (SF)	+	+	+	+
3rd Leaf blade (SF)	+	+	+	+
4th Leaf blade (SF)	+	+	+	+
1st Leaf petiole (SF)	+	+	+	+
2nd Leaf petiole (SF)	+	+	+	+
3rd Leaf petiole (SF)	+	+	+	+
4th Leaf petiole (SF)	+	+	+	+
Leaf bud (FF)	+	+	+	+
1st Leaf blade (FF)	+	+	+	+
2nd Leaf blade (FF)	+	+	+	+
3rd Leaf blade (FF)	+	+	+	+
4th Leaf blade (FF)	+	+	+	+
1st Leaf petiole (FF)	+	+	+	+
2nd Leaf petiole (FF)	+	+	+	+
3rd Leaf petiole (FF)	+	+	+	+
4th Leaf petiole (FF)	+	+	+	+

(+) = Presence of chemical constituents, (-) = absence of chemical constituents; F- Flavonoids; S- Saponins; St- Steroid glycosides; T- Tannins; SF: Soft Flesh; FF: Firm Flesh

3.2. Functional Properties

The total phenolic content (TPC) determined in different leaf positions of two types of *A. heterophyllus* is demonstrated in Table 2. TPC of leaf buds and leaf blades of *A. heterophyllus* at different leaf positions varied between 16.79 ± 0.33 to 19.09 ± 0.31 mg GAE/g DW. The significantly highest TPC (19.09 ± 0.31 GAE /g DW) was recorded in the second leaf blade of soft flesh type whereas, high TPCs (18.46 ± 0.39 and 18.29 ± 0.36 GAE /g DW) were recorded in the first and second leaf blades of firm flesh type respectively. Significantly lowest TPC was recorded in the fourth leaf blade of both types. TPC of leaf petioles of *A. heterophyllus* at different leaf positions varied from 13.02 ± 0.00 to 18.47 ± 0.41 mg GAE/g DW. These results were agreed with Kurian *et al*. [7], who reported that TPC in jackfruit leaf petioles was 24.23 ± 1.04 mg GAE/g DW. The significantly highest TPC (19.09 ± 0.31 GAE /g DW) was recorded in the fourth petiole of the soft flesh type, whereas the second petiole of the firm flesh type (15.17 ± 0.58 GAE /g DW). Kurian *et al*. [7] reported that traditionally the decoction of the mature leaf petiole of jackfruit is used in various ailments because of this high content of bioactive compounds. Moreover, leaf buds and blades of both *A. heterophyllus* types have significantly higher TPC than leaf petioles.

When considering the leaf bud and blades, as similar to the TPC, significantly high TFCs (326.19 ± 8.67 and 268.14 ± 11.56 mg RE/g DW) were recorded in the second leaf blade of soft flesh type and first leaf blade of firm flesh type, respectively (Table 2). Significantly lowest TFC was recorded in the fourth leaf blade of both types, and there was no significant difference between the fourth leaf blade and the leaf bud of the soft flesh type. When considering the leaf petioles, significantly high TFCs (208.42 ± 6.29 and 388.14 ± 20.57 mg RE/g DW) were also recorded in the second petiole of firm flesh type similar to the TPC. However, it varied in soft flesh type as the first petiole (Table 2).

When considering the leaf bud and blades, as similar to the TPC and TFC, significantly high TACs (147.04 ± 3.71 and 119.55 ± 12.74 mg TE/g DW) were recorded in the second leaf blade of soft flesh type and first leaf blade of firm flesh type respectively (Table 2). Significantly lowest TAC was recorded in the fourth leaf blade of both types. Moreover, there was no significant difference between the fourth leaf blade and the firm flesh-type leaf bud. When considering the leaf petioles, as similar to the TPC and TFC, significantly high TACs (149.47 ± 6.05 and 94.28 ± 9.05 mg TE/g DW) were also recorded in fourth leaf petiole of soft flesh type and second petiole of firm flesh type respectively (Table 2).

The results of the present study revealed that all the tested leaf positions of both *A. heterophyllus* types contained remarkable amounts of bioactive compounds and antioxidant capacity. However, there was a slight reduction in TAC, TPC and TFC of leaf blades with the maturity.

3.3. Correlation of TAC with TPC and TFC

The findings of this study showed positive correlations of TAC with TFC (R2 = 0.7828) and TPC (R2 = 0.4364). These positive correlations suggest that the phenolic and flavonoid components contribute significantly to the antioxidant capacity of different leaf positions of *A. heterophyllus*

Table 2. Total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC) of different leaf positions of soft and firm flesh trees of *Artocarpus heterophyllus*

Type	Plant material	TPC (mg GAE /g DW)	TFC (mg RE /g DW)	TAC (mg TE / g DW)
Soft flesh	Leaf bud	17.97 ± 0.40^{abc}	242.58 ± 10.83^{gh}	98.52 ± 9.42^{cd}
	1st Leaf blade	18.72 ± 0.23^{ab}	296.47 ± 17.92^{cde}	121.23 ± 8.84^{bc}
	2nd Leaf blade	19.09 ± 0.31^{a}	326.19 ± 8.67^{bc}	147.04 ± 3.71^{a}
	3rd Leaf blade	18.20 ± 0.13^{ab}	265.08 ± 8.21^{fg}	113.36 ± 6.98^{cd}
	4th Leaf blade	17.85 ± 0.37^{abc}	250.08 ± 7.95^{gh}	93.81 ± 5.27^{d}
	1st Leaf petiole	16.16 ± 0.34^{de}	388.14 ± 20.57^{a}	119.94 ± 10.51^{bc}
	2nd Leaf petiole	17.54 ± 1.23^{bcd}	292.58 ± 9.61^{def}	147.53 ± 5.28^{a}
	3rd Leaf petiole	14.98 ± 0.28^{ef}	300.36 ± 10.88^{cd}	140.49 ± 8.52^{ab}
	4th Leaf petiole	18.47 ± 0.41^{ab}	338.97 ± 7.09^{b}	149.47 ± 6.05^{a}
Firm flesh	Leaf bud	16.79 ± 0.33^{cd}	182.31 ± 11.31^{j}	89.68 ± 10.99^{d}
	1st Leaf blade	18.46 ± 0.39^{ab}	268.14 ± 11.56^{efg}	119.55 ± 12.74^{bc}
	2nd Leaf blade	18.29 ± 0.36^{ab}	233.14 ± 4.59^{hi}	111.49 ± 9.80^{cd}
	3rd Leaf blade	18.15 ± 0.66^{abc}	230.08 ± 3.63^{hi}	108.26 ± 10.90^{cd}
	4th Leaf blade	17.75 ± 0.21^{abc}	202.03 ± 11.82^{ij}	92.85 ± 3.22^{d}
	1st Leaf petiole	13.02 ± 0.00^{g}	117.29 ± 5.76^{k}	55.14 ± 5.55^{e}
	2nd Leaf petiole	15.17 ± 0.58^{ef}	208.42 ± 6.29^{ij}	94.28 ± 9.05^{d}
	3rd Leaf petiole	14.03 ± 0.28^{fg}	102.74 ± 2.95^{k}	40.74 ± 2.15^{e}
	4th Leaf petiole	14.21 ± 0.11^{fg}	114.29 ± 5.80^{k}	50.67 ± 2.91^{e}

Within columns, values followed by different superscript letters are significantly different at P<0.05, TE: Trolox Equivalent; GAE: Gallic Acid Equivalent; RE: Rutin Equivalent; DW: Dry Weight

3.4. Physiochemical Properties

The quality of jackfruit leaf-based products may depend mainly on their phytochemical and physiochemical properties. The results of moisture, ash and crude protein contents are presented in Table 3. The percentage of moisture, ash and crude protein contents of different leaf positions of *A. heterophyllus* types were varied within the ranges of 55.84 ± 1.58% to 74.12 ± 1.30%, 7.79 ± 0.28% to 19.78 ± 0.20% and 7.24 ± 0.20% to 16.81 ± 0.18% respectively. When considering the leaf bud and leaf blades, the highest crude ash content was reported in the leaf bud of both *A. heterophyllus* types. However, leaf petioles' moisture and ash content were significantly higher than leaf buds and leaf blades of both *A. heterophyllus* types. When considering the leaf bud and leaf blades, the highest crude protein content was reported in the leaf bud of both *A. heterophyllus* types. The crude protein contents of leaf buds and leaf blades were significantly higher than leaf petioles of both *A. heterophyllus* types. The moisture content of the leaves was approximately similar to the previous study [14]. However, ash and crude protein contents presented in our study were higher than that reported by Amadi *et al.* [14], who reported 2.53% ash content and 1.19% protein content in jackfruit leaves. Variations of physicochemical properties could result from various geographical locations, including soil type. However, Brion-Espinoza *et al.* [15] reported 7.64 ± 0.12% protein content in fresh leaves of jackfruit.

Table 3. Proximate composition of different leaf positions of soft and firm flesh trees of *Artocarpus heterophyllus*

Type	Plant material	Moisture (%)	Crude ash (%)	Crude protein (%)
Soft flesh	Leaf bud	68.30 ± 1.15^{cde}	11.52 ± 0.61^{fg}	16.81 ± 0.18^{a}
	1st Leaf blade	68.88 ± 0.12^{cde}	7.79 ± 0.28^{i}	15.12 ± 0.27^{c}
	2nd Leaf blade	65.73 ± 2.02^{ef}	8.50 ± 0.06^{hi}	14.12 ± 0.36^{d}
	3rd Leaf blade	58.05 ± 0.84^{g}	8.14 ± 0.08^{hi}	13.72 ± 0.10^{d}
	4th Leaf blade	55.84 ± 1.58^{g}	10.54 ± 0.62^{g}	15.35 ± 0.10^{c}
	1st Leaf petiole	73.34 ± 1.70^{a}	12.52 ± 0.26^{ef}	8.99 ± 0.36^{ef}
	2nd Leaf petiole	73.96 ± 0.76^{a}	11.84 ± 0.37^{f}	8.35 ± 0.27^{fg}
	3rd Leaf petiole	72.28 ± 1.59^{ab}	13.22 ± 0.27^{de}	7.53 ± 0.00^{hi}
	4th Leaf petiole	72.96 ± 1.04^{ab}	14.15 ± 0.50^{cd}	8.00 ± 0.10^{gh}
Firm flesh	Leaf bud	69.97 ± 0.34^{bcd}	14.91 ± 0.82^{c}	16.11 ± 0.30^{b}
	1st Leaf blade	71.04 ± 1.01^{abc}	9.13 ± 0.08^{h}	15.52 ± 0.20^{bc}
	2nd Leaf blade	67.32 ± 0.38^{de}	9.04 ± 0.15^{h}	14.24 ± 0.27^{d}
	3rd Leaf blade	63.29 ± 0.98^{f}	11.35 ± 0.19^{fg}	14.01 ± 0.18^{d}
	4th Leaf blade	58.20 ± 0.72^{g}	11.91 ± 0.16^{f}	15.00 ± 0.27^{c}
	1st Leaf petiole	73.88 ± 0.81^{a}	16.66 ± 0.36^{b}	9.28 ± 0.18^{e}
	2nd Leaf petiole	73.28 ± 0.30^{ab}	17.49 ± 0.58^{b}	8.35 ± 0.10^{fg}
	3rd Leaf petiole	74.01 ± 0.63^{a}	19.14 ± 0.50^{a}	7.24 ± 0.20^{i}
	4th Leaf petiole	74.12 ± 1.30^{a}	19.78 ± 0.20^{a}	8.99 ± 0.10^{ef}

Within columns, values followed by different superscript letters are significantly different at P<0.05

4. Conclusions

The results of the present study conclude that all the tested leaf positions of both *A. heterophyllus* types contained marked amounts of bioactive compounds and antioxidant capacity. The leaves of the soft flesh type consist of more bioactive compounds and antioxidant capacity compared to the firm flesh type. Our results suggest that, thus, jackfruit leaves of both soft and flesh varieties of jackfruit could be used for the preparation of jackfruit leaf-based value-added products.

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