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Proximate Composition, Bioactive Compounds and Total Antioxidant Capacity of Immature Leaves of Selected Fruit and Vegetable Crops

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PROXIMATE COMPOSITION, BIOACTIVE COMPOUNDS AND TOTAL ANTIOXIDANT CAPACITY OF IMMATURE LEAVES OF SELECTED FRUIT AND VEGETABLE CROPS

Abstract: The present study was undertaken to quantify the total phenolic content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC), proximate composition and chlorophyll content of immature leaves of seven selected crops namely *Manihot esculenta* (cassava), *Ipomoea batatas* (sweet potato), *Cucurbita maxima* (pumpkin), *Psophocarpus tetragonolobus* (winged bean), *Lagenaria siceraria* (bottle gourd), *Spondias dulcis* (*ambarella*) and *Passiflora edulis* (passion fruit). TPC, TFC and TAC were determined using folin-ciocalteu method, colorimetric method and ferric iron reducing antioxidant power (FRAP) respectively. The moisture, ash and protein were analyzed using the methods reported by the Association of Official Analytical Communities (AOAC). The highest TPC (19.04 ± 0.76 mg GAE /g DW) and TAC (115.94 ± 6.27 mg TE / g DW) were recorded in Manihot esculenta leaves. Significantly, the highest TFC (134.02 ± 3.25 mg RE /g DW) and ash content (10.81 ± 0.21 %) were recorded in *Ipomoea batatas* leaves. The leaves of *Psophocarpus tetragonolobus* had the highest protein content (36.71 ± 0.10 %) and total chlorophyll content (2.60 ± 0.01 mg / g FW). According to the results, it can be concluded that all tested immature leaves contained marked amounts of bioactive compounds and total antioxidant capacity.

Keywords: Antioxidant capacity, Immature leaves, Phenolic, Proximate composition

Introduction

Currently, due to the economic inflation in Sri Lanka, the prices of all goods have been increased. As well as with the economic downturn and job losses brought on by the COVID-19 epidemic, there will be a severe food insecurity in Sri Lanka (Sinha, 2021). The food insecurity causes to low diet quality and it interferes with the daily food requirement of the people.

With the increase of vegetable prices, people are now motivated to grow and eat their desired vegetable crops in their own backyards. Consumption of fruits and vegetables grown at home has significantly increased (Sooriyaarachchi *et al*., 2022). As leafy vegetables, they mainly grow Gotukola, Mukunuwenna and Sarana like crops. But in some areas, people consume the immature leaves of crops grown as vegetable or fruit crops like Winged bean, Cassava and Passion fruit. Their main products are pod, yam or a fruit. The by-product is immature leaves as leafy vegetables. People can meet their vegetable or fruit requirement and leafy vegetable requirement from same crop.

The cassava is mainly cultivated as a tuber crop but their immature leaves can be consumed. Interesting sources of nutrients like fiber, protein, vitamin A, and vitamin C are found in immature cassava leaves (Lancaster and Brooks, 1983).

The sweet potato leaves are significant source of polyphenols with a range of bioactive compounds (Chao *et al*., 2014). As the primary phenolic compounds in sweet potato leaves, caffeic acid and caffeoylquinic acid derivatives are identified (Luo *et al*., 2013). In addition to that, flavonoids, alkaloids, saponins, coumarins and tannins like compounds are in sweet potato leaves that help to antioxidant activity (Nguyen *et al*., 2021).

Pumpkin leaves have great nutritional, therapeutic, and industrial values because they are high in protein (9%), fat (18%), and vitamins (20%) (Sharma *et al*., 2019). The leaf extract of pumpkin is an excellent source of iron, which raises the blood's level of hemoglobin (Roughan, 1970).

The bottle gourd is a member of the genus Lagenaria. Its' fruits and aerial sections are both frequently eaten as vegetables. Leaves of bottle gourd contain cucurbitacin B. The leaves of bottle gourd have been used to alleviate baldness and headaches (Tyagi *et al*., 2017).

The winged bean is a tropical leguminous plant. This plant has vitamins A, C, calcium, iron, proteins, and lipids and leaves have excellent antioxidant activities (Nagarajan and Rao, 2019).

When considering the ambarella (*Spondias dulcis)*, it is another crop type, which consumed as fruit or vegetable. The research was conducted to find the antidiabetic effect of leaves (Mohiuddin *et al*., 2016).

Sunitha and Devaki (2009) had identified antioxidants of passion fruit leaves.

People who consume foods high in antioxidants may have a lower chance of developing a wide range of diseases including cancer, cardiovascular disease, chronic diseases, and aging according to experimental and epidemiological researches (Linn and Myint, 2018).

Though several isolated studies have been conducted to determine proximate composition, bioactive compounds and antioxidant capacity of leaves of above mention crops a comprehensive study on proximate composition, bioactive compounds and antioxidant capacity of immature leaves of above mention crops is lacking.

As above details, there are little number of studies were conducted to evaluate antioxidant capacities and bioactive compounds in immature leaves of these selected fruits and vegetables. Therefore, this study will be conducted to determine proximate composition, bioactive compounds and antioxidant capacity of immature leaves of passion fruit (*Passiflora edulis*), cassava (*Manihot esculenta*), winged bean (*Psophocarpus tetragonolobus*), *ambarella* (*Spondias dulcis*), pumpkin (*Cucurbita maxima*), sweet potato (*Ipomoea batatas*) and bottle guard (*Lagenaria siceraria*).

Methodology

Location

This study was carried out in the laboratory of Department of Plantation Management, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP).

Sample Collection

The immature leaves of Manihot esculenta, Ipomoea batatas, Cucurbita maxima, Psophocarpus tetragonolobus, Lagenaria siceraria, Spondias dulcis, Passiflora edulis were collected in the month of

December 2022 from Makandura area (Table 1). The homogeneous representative samples were collected in each type of crops and mixed to make composite samples.

Table 1. Common name and crop specification of samples

Scientific name	Common name	Crop specification
Passiflora edulis	Passion fruit leaves	Red passion fruit
Spondias dulcis	Ambarella leaves	-
Manihot esculenta	Cassava leaves	Suranimala
Psophocarpus tetragonolobus	Winged bean leaves	Green winged been
Cucurbita maxima	Pumpkin leaves	Malbaro variety
Ipomoea batatas	Sweet potato leaves	White flesh coloured yam
Lagenaria siceraria	Bottle gourd leaves	Diya labu

Sample Preparation

The fresh immature leaf samples were washed and cut into small pieces and air-dried for three days at room temperature (28 ^{0}C ± 2). Then samples were powdered using coffee grinder. The leaf powder was sieved with 0.25 mm mesh. Ground samples were packed in polythene bags and stored in a refrigerator until analysis (Nipunika *et al*., 2022).

Extraction of phytochemicals

The powdered samples (0.5 g) were weighed into 15 ml centrifuge tubes. Then 5 ml of 80% methanol was added and vortexed for 15 min. After that, it was placed in a water bath at 60 ^{0}C for 40 min. The vortex procedure was repeated at 10 min intervals. Then, samples were centrifuged at 4,000 rpm for 5 min. The supernatant was decanted into a new 15 ml centrifuge tube and the remaining was re-extracted with 5 ml of 80% methanol. The supernatants were pooled and stored at -20 ^{0}C prior to analysis (Nipunika *et al*., 2022).

Preparation of Pigment Stock Solution for Chlorophyll Determination

Approximately 0.25 g of fresh leaf material was measured separately from each plant species. Leaf tissues were homogenized thoroughly by grinding it in 10 ml of 80% (v/v) acetone, using the mortar and pestle. The homogenate was filtered into stock flask. Then, the tissue debris were scraped back to the mortar and pestle. Grinding and filtering were repeated once to extract all the pigments. All the filtrates were combined and mixed well. After, the final volume was adjusted to 50 ml with 80% acetone (Udadeniya *et al*.,2016).

Determination of Functional Properties

Total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC) were determined by modified folin-ciocalteu method (Abeysinghe *et al*., 2007), a colorimetric method

(Liu *et al*., 2002) and Ferric Reducing Antioxidant Power (FRAP) assay (Benzie and Strain, 1996) respectively.

Determination of Chlorophyll Content

The chlorophyll content was determined by an absorption spectrum of chlorophyll in the immature leaves using the spectrophotometer as described by Udadeniya *et al*. (2016).

Determination of Proximate Composition

The moisture, crude protein and ash contents of samples were analyzed using the methods reported by the Association of Official Analytical Chemists (AOAC, 1990).

Statistical Analysis

Parametric variables were analyzed using analysis of variance (ANOVA) followed by Turkey Multiple Range Test using SAS (SAS Version 9.5).

Results and discussion

Functional Properties

The total phenolic content (TPC) varied from 0.98 ± 0.03 to 19.04 ± 0.76 mg GAE /g DW (Table 2). Significantly, the highest TPC (19.04 ± 0.76 mg GAE /g DW) was observed in *Manihot esculenta* leaves whereas, significantly the low TPCs (0.98 ± 0.03 mg GAE /g DW and 1.79 ± 0.09 mg GAE /g DW) were recorded in leaves of *Lagenaria siceraria* and *Passiflora edulis* respectively.

There was a significant difference among the TFC of tested immature leaf extracts. (Table 2). Significantly, highest TFC (134.02 ± 3.25 mg RE /g DW) was recorded in immature leaves of *Ipomoea batatas*.

Total antioxidant capacity (TAC) of tested immature leaf extracts were ranged from 4.18 ± 0.50 to 115.94 ± 6.27 mg TE / g DW (Table 2). Significantly, the highest TAC was observed in immature leaves of *Manihot esculenta* (115.94 ± 6.27 mg TE / g DW). This may be probably due to high chlorophyll content in cassava leaves (Table 3). Significantly, low TACs were observed in immature leaves of *Cucurbita maxima* and *Psophocarpus tetragonolobus* (4.18 ± 0.50 mg TE / g DW and 4.96 ± 0.38 mg TE / g DW) respectively. Results obtained from this study agreed with the findings of Linn and Myint (2018) which reported that cassava leaves have potent antioxidant activity. The ascending order of TAC of the tested immature leaves are as follows; *Cucurbita maxima* < *Psophocarpus tetragonolobus* < *Passiflora edulis* < *Lagenaria siceraria* < *Spondias dulcis* < *Ipomoea batatas* < *Manihot esculenta*. According to Nurrofingah *et al*. (2019), the antioxidant capacity of sweet potato leaves is lower than cassava leaves and results obtained from this study is also agreed with that finding. Further, this study revealed that there are positive correlations of TPC (R2 = 0.69) and TFC (R2 = 0.27) with TAC.

Table 2. Total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC), of immature leaves of selected fruit, vegetable and tuber crops

Plant material	TPC (mg GAE /g DW)	TFC (mg RE /g DW)	TAC (mg TE / g DW)
Sweet potato	16.69 ± 0.25^{b}	134.02 ± 3.25^{a}	50.84 ± 2.23^{b}
Cassava	19.04 ± 0.76^{a}	63.68 ± 1.33^{c}	115.94 ± 6.27^{a}
Pumpkin	3.96 ± 0.28^{d}	3.96 ± 0.25^{e}	4.18 ± 0.50^{e}
Winged bean	4.06 ± 0.18^{d}	3.74 ± 0.67^{e}	4.96 ± 0.38^{e}
Bottle gourd	0.98 ± 0.03^{e}	8.96 ± 0.59^{e}	27.98 ± 2.31^{cd}
Ambarella	5.01 ± 0.13^{c}	73.96 ± 5^{b}	33.29 ± 1.77^{c}
Passion fruit	1.79 ± 0.09^{e}	24.41 ± 1.11^{d}	22.41 ± 2.23^{d}

Mean denoted by the same letters in a column represent non-significant differences (P>0.05); TE= Trolox equivalent; GAE= Gallic acid equivalent; RE= Rutin equivalent; DW= Dry weight

Total Chlorophyll

Significantly, highest chlorophyll was obtained in immature leaves of winged bean (2.60 ± 0.01 mg / g FW) whereas second highest chlorophyll content was obtained in cassava leaves (2.35 ± 0.11 mg / g FW). Zepka *et al*. (2019) mentions that chlorophyll a (Ch a) and chlorophyll b (Ch b) have anti-mutagenic and antioxidant activity like bioactive properties.

Table 3. Chlorophyll a (Ch a), chlorophyll b (Ch b) and total chlorophyll content (Total Ch) of immature leaves of selected fruit, vegetable and tuber crops

Plant material	Ch a (mg / g FW)	Ch b (mg / g FW)	Total Ch (mg / g FW)
Sweet potato	0.77 ± 0.00^{e}	0.45 ± 0.00^{d}	1.22 ± 0.00^{d}
Cassava	1.75 ± 0.05^{b}	0.61 ± 0.06^{c}	2.35 ± 0.11^{b}
Pumpkin	0.96 ± 0.00^{d}	0.84 ± 0.01^{a}	1.80 ± 0.01^{c}
Winged bean	1.84 ± 0.01^{a}	0.76 ± 0.01^{b}	2.60 ± 0.01^{a}
Bottle gourd	0.98 ± 0.03^{d}	0.34 ± 0.01^{e}	1.32 ± 0.04^{d}
Ambarella	0.30 ± 0.03^{f}	0.21 ± 0.02^{f}	0.52 ± 0.02^{e}
Passion fruit	1.31 ± 0.04^{c}	0.48 ± 0.02^{d}	1.79 ± 0.03^{c}

Mean denoted by the same letters in a column represent non-significant differences (P>0.05); FW= fresh weight

Proximate Composition

The moisture content values obtained in this study ranged from 78.88 ± 0.55% to 84.42 ± 0.32% (Table 4). The significantly higher moisture contents were observed in the sweet potato leaves (84.42 ± 0.32%), bottle gourd (84.17 ± 0.15%) and *ambarella* (84.06 ± 0.18%). The winged bean leaves showed the significantly lowest moisture content (78.88 ± 0.55%).

When comparing ash contents, significantly highest ash content was observed in immature leaves of sweet potato (10.81 ± 0.21%) (Table 4) and this finding was agreed with the findings of Nkongho *et al.* (2014) which has reported an ash content of sweet potato leaves in 9% to 14% range. The plants included a good amount of minerals because the ash content is a reflection of the quantity of mineral elements found in the samples (Aborisade *et al*., 2017).

There was a significant difference among the protein content of tested leaf samples and it ranged from 20.54 ± 0.10% to 36.71 ± 0.10% (Table 4). Significantly highest protein content was obtained in immature leaves of winged bean (36.71 ± 0.10%) and this result agreed with the findings of Okezie and Martin (1980) which has reported protein content of winged bean leaves in 27% to 36% range. Second highest protein content was recorded in passion fruit (30.70 ± 0.20%) and it was not significantly different with protein content of immature leaves of pumpkin (30.52 ± 0.10%). The lower protein contents were recorded in immature leaves of sweet potato (20.54 ± 0.10%), cassava (21.14 ± 0.19%) and *ambarella* (21.77 ± 0.51%). Nkongho *et al*. (2014) has reported the crude protein in young sweet potato leaves in 15% – 27% range. According to Nassar and Marques (2006), the protein content of cassava leaves ranged from 21% – 32% and it depends on the cassava variety.

Table 4. Moisture, ash and protein content of immature leaves of selected fruit, vegetable and tuber crops

Plant material	Moisture (%)	Ash (%)	Protein (%)
Sweet potato	84.42 ± 0.32^{a}	10.81 ± 0.21^{a}	20.54 ± 0.10^{e}
Cassava	81.31 ± 0.58^{c}	5.07 ± 0.58^{cd}	21.14 ± 0.19^{de}
Pumpkin	81.19 ± 0.16^{c}	4.04 ± 0.38^{e}	30.52 ± 0.10^{b}
Winged bean	78.88 ± 0.55^{d}	3.57 ± 0.23^{e}	36.71 ± 0.10^{a}
Bottle gourd	84.17 ± 0.15^{a}	4.47 ± 0.42^{de}	28.25 ± 0.10^{c}
Ambarella	84.06 ± 0.18^{a}	5.86 ± 0.12^{c}	21.77 ± 0.51^{d}
Passion fruit	82.67 ± 0.40^{b}	8.73 ± 0.42^{b}	30.70 ± 0.20^{b}

Mean denoted by the same letters in a column represent non-significant differences (P>0.05)

Conclusions

The immature leaves of sweet potato and cassava are rich in TPC and TAC. The sweet potato leaves contain good amount of minerals. Further, all tested phytochemicals are contained in leaves of cassava and sweet potato. The winged bean leaves are the good source of protein. According to the results, all immature leaves contain marked amounts of ash, protein, bioactive compounds and antioxidant capacity. Therefore, all tested immature leaves can be consumed as leafy vegetables.

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