COMPARISON OF PROXIMATE COMPOSITION AND FIBER CONTENT BETWEEN VARIETIES OF SRI LANKAN GREEN LEAFY VEGETABLES *Ipomea aquatica*, *Centella asiatica* AND *Sesbania grandiflora*

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Introduction

Nutrition stands as vital for human existence. Despite its critical importance, nations with lower and middle incomes, such as Sri Lanka, can be exceptionally susceptible to food insecurity, especially in the face of economic crises right after the pandemic. Many Sri Lankans are consequently leading to malnutrition, with considerable difficulty in securing an ample supply of nutritious food during this crisis period. Thus, Sri Lanka consists of cost effective, readily available, nutrient-dense, and locally sourced food.

Green leafy vegetables (GLVs) comprise abundant essential dietary components, including vital macronutrients [1], [2]. The Dietary Guidelines for Americans recommends five servings of vegetables each day, with one of those servings specifically emphasizing GLVs [HHS/USDA 2020-2025]. Notably, the traditional Sri Lankan diet mainly comprises GLVs. Sri Lanka with its tropical climate abides by a diverse range of GLVs. Consequently, exploring the nutritional value of the GLVs, particularly as a sustainable approach to combat malnutrition, is important and justifies further investigation.

The nutrient constituents of GLVs can vary depending on several factors including environmental factors, agricultural practices and intrinsic factors such as species and variety. Numerous studies have demonstrated the fluctuations in the nutritional profiles of species, yet inquiries related to varieties are scarce. This study was carried out to study the comparative proximate analysis (the moisture, ash, carbohydrate, protein, and fat contents) and fiber content between two varieties of selected three GLV species; *Ipomea aquatica*, *Centella asiatica* and *Sesbania grandiflora* (Figure 1), which are commonly consumed in Sri Lanka. *I. aquatica* samples were collected from the home gardens of Jaffna and *S. grandiflora* and *C. asiatica* samples were collected from Kalutara. Two different varieties of each species were acquired from similar geographical locations, grown under similar growth conditions such as light, water, nutrients, and temperature, and were identified based on morphological characteristics.

The knowledge from this study holds the potential to refine the selection processes of GLVs in agriculture. Furthermore, this study paves the way to expand the GLV applications within the food industry leading to amplified nutritional advantages.

Material and Methods

Materials

Two morphologically different varieties of each, *I. aquatica*, *C. asiatica,* and *S. grandiflora* were selected. The plant specimens were taxonomically identified using the online herbarium of the Faculty of Agriculture, University of Ruhuna. Chemical reagents, sulfuric acid (H_2SO_4), and petroleum ether were obtained by Breckland Scientific™, UK. Sodium hydroxide (NaOH), mercuric oxide (HgO), potassium sulfate (K_2SO_4), sodium thiosulfate ($Na_2S_2O_3$) and Zinc (Zn) granules were obtained from Merck™, Germany.

Sample Preparation

GLV samples were cleaned well with tap water and distilled water to remove dust, mud, and other possible impurities. The inedible parts were removed. Samples were air dried at room temperature under shade, to remove excess water. Then dried in a hot air oven at 45 ˚C to obtain a constant weight and ground to fine powder.

Determination of Moisture Content

Moisture content was determined using the loss on drying approved by the AOAC methods (2009) [2]. 2 g amount of the sample was dried in a hot air oven at 95 – 100 ˚C to a constant weight. The moisture content was calculated using the following equation. (W_d: weight loss on drying, W_s: weight of the sample)

$$Moisture\ Content\ \% = \frac{W_d \times 100}{W_s}$$

Determination of Ash Content

Protein content was determined using the dry ashing method approved by AOAC methods (2009) [2]. 3 g amount of the sample was ashed in a muffle furnace (BIOBASE) at 600 ˚C for 2 hours. The ash content was calculated using the following equation. (W_a: weight loss on ashing, W_s: weight of the sample

$$Ash\ Content\ \% = \frac{(W_s - W_a) \times 100}{W_s}$$

Determination of Crude Protein Content

Protein content was determined using the Kjeldahl method approved by AOAC method (2009) [2]. First, 1.0 g of homogenous sample powder was boiled briskly in 12.5 mL of concentrated H_2SO_4 with 0.35 g HgO and 7.5 g K_2SO_4 in a digestion flask until frothing ceased and the solution was clear. The solution was cooled and diluted with 100 mL of distilled water and mixed with $Na_2S_2O_3$ solution ($Na_2S_2O_3$ 2 g dissolved in 25 mL of distilled water). A few Zn granules and a layer of NaOH were added without agitation. The flask was immediately connected to the distillation unit and released NH_3 was collected by immersing the condenser tip in a standard H_2SO_4 solution. The excess standard acid was determined by titration using a standard NaOH solution. A blank analysis was carried out to

remove possible errors from reagents. The crude protein content was calculated using the following equation (V: volume, M: molarity, W: weight of the sample, 4.64 - protein conversion factor).

$$Crude\ Protein\ \% = \frac{[(V\ H_2SO_4\ \times 2\ \times M\ H_2SO_4) - (V\ NaOH\ \times M\ NaOH)] \times 1.4007 \times 100 \times 4.64}{W}$$

Determination of Crude Fat Content

Crude fat content was determined using the Soxhlet extraction method approved by the AOAC method (2009) [2]. First, 3.0 g of the homogenous sample powder was added to a cellulose thimble and extracted with petroleum ether for 5 hours using the Soxhlet apparatus. The solvent was evaporated using a rotary evaporator (PHOENIX). The flask was placed in a hot air oven at 110 ˚C for 30 min and cooled in a desiccator. The weight of the flask with fat was measured. A blank analysis was carried out to remove possible errors from reagents. The crude fat content was calculated using the following equation (W_a: weight of the flask with fat, W_b: weight of the flask without fat, W: weight of the sample).

$$Crude\ Fat\ \% = \frac{(W_a - W_b)100}{W}$$

Determination of Crude Fiber Content

The crude fiber content was determined using the acid and base digestion method [3]. First, 1.0 g of the homogenous sample powder was boiled in 100 mL of 0.128 M H_2SO_4 for 30 min and the residues were filtered using muslin cloth and was washed three times with hot distilled water. Then the residues were boiled in 0.125 M NaOH for 30 min, filtered with muslin cloth and washed with hot distilled water. The residues were further washed with acetone and oven-dried at 105 °C to constant weight and weighed. Then ashed at 500 °C for 2 hours using a muffle furnace and the ash was weighed. The crude fiber content was calculated using the following equation. (W_f: weight of fiber, W_a: weight of ash, W: weight of the sample).

$$Crude\ Fiber\ \% = \frac{\left(W_f - W_a\right)100}{W}$$

Determination of Carbohydrate Content

Carbohydrate content was determined using a difference method [4] by subtracting the sum of the per cent of protein, moisture, fat and ash from 100.

$$Carbohydrate\ \% = 100 - (Moistur + Ash + Protein + Fat)$$

Statistical Analysis

All the experiments were designed with triplicates and expressed as mean ± standard deviation (SD). The data for all determinations were subjected to a one-way analysis of variance ANOVA test using IBM SPSS Statistics Data Editor. A

Tuckey post hoc test was carried out to detect significant differences between the selected two varieties in each aspect. Differences were considered statistically significantly different if the probability values were less than 0.05; the standard alpha level (P<0.05).

Results and Discussion

Two species, *I. aquatica* and *C. asiatica* showed pronounced morphological differences in their varieties (Figure 1). Two varieties of *I. aquatica* (Kankun) were morphologically different from the size of the leaf lamina where a wider leaf lamina was observed in V1 (variety 1). *C. asiatica,* (common Gotukola) V1 was easily discriminated against *C. asiatica* (Weda Gotukola) V2 (variety 2) by its growth habit and size of leaves. *C. asiatica* V1 was bush type in growth habit with medium size leaves while *C. asiatica* V2 was vine-type with small leaves. No pronounced morphological differences were observed in the leaves of both *S. grandiflora* varieties (Figure 1). However, *S. grandiflora* V1 bears white flowers frequently, while V2 is rarely flowering white flowers and is called the "Haritha" variety.

The proximate composition and the fiber contents of six green vegetable samples were analyzed and the results are elaborated in Table 1. The moisture content between the two varieties of each species was not statistically significantly different. The highest moisture content was observed in V1 *I. aquatica*, while the lowest was in V2 *S. grandiflora*. The two varieties of *C. asiatica* showed a statistically significant difference in ash content, where the highest and lowest values were observed in *S. grandiflora* V2 (2.97±0.13) and *I. aquatica* V2 (1.51±0.02) respectively. Only the two varieties of *S. grandiflora* showed statistically significant differences in the aspect of carbohydrate content. The highest and the lowest carbohydrate values were observed in *S. grandiflora* V2 (27.78±1.19) and *I. aquatica* V1 (5.40±0.51) respectively. The crude protein content was statistically significantly different between the varieties of *S. grandiflora*. The two varieties of *I. aquatica* and *C. asiatica* were not significantly different. The highest and the lowest crude protein contents were observed in V1 *S. grandiflora* and V2 of *C. asiatica* respectively. The crude fat content between the two varieties of each species was not statistically significantly different. The highest and a similar crude fat content was observed in V1 and V2 of *S. grandiflora*. The lowest crude fat content (0.28±0.09) was observed in V2 of *I. aquatica*. All three species showed statistically significant differences in two varieties for the crude fiber content. The highest crude fiber content was observed in V2 of *C. asiatica* while the lowest was V1 of *I. aquatica*.

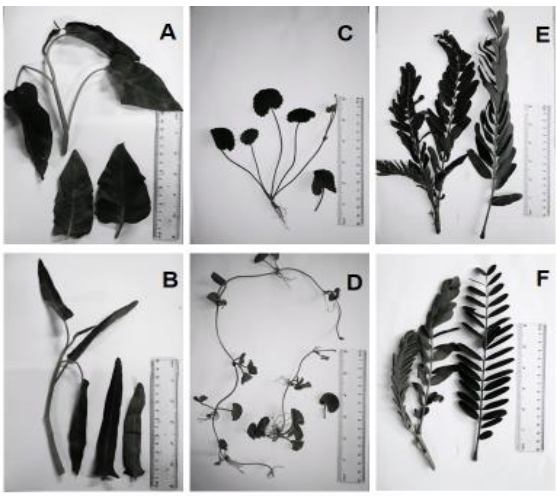

Figure 1. (A) *I. aquatica* (Kankung) Variety 1, (B) *I. aquatica* (Kankung) Variety 2, (C) *C. asiatica* (Common Gotukola) Variety 1, (D) *C. asiatica* (Weda Gotukola) Variety 2, (E), *S. grandiflora* (Kathurumurunga) Variety 1, (F) *S. grandiflora* (Kathurumurunga) Variety 2 (Haritha variety).

Table 1. The proximate composition and the fiber content of the tested leafy vegetable varieties.

Green Leafy Veget-able	Variety	Moisture (%)	Ash (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Carbohy-drate (%)
I. aquatica	V1	89.99±0.38^{c}	1.52±0.07^{a}	2.45±0.19^{ab}	0.50±0.09^{ab}	2.93±0.38^{a}	5.40±0.51^{a}
	V2	88.73±1.11^{c}	1.51±0.02^{a}	3.44±0.28^{b}	0.28±0.09^{a}	5.91±0.36^{b}	5.86±1.00^{a}
C. asiatica	V1	80.00±0.52^{b}	2.05±0.07^{b}	2.13±0.66^{a}	0.62±0.07^{b}	13.44±0.66^{b}	15.07±1.31^{b}
	V2	77.32±3.71^{b}	1.73±0.07^{a}	1.90±0.41^{a}	0.52±0.02^{ab}	17.46±0.85^{c}	18.43±3.37^{b}
S. grandiflora	V1	69.77±2.42^{a}	2.88±0.19^{c}	6.51±0.36^{c}	1.48±0.17^{c}	11.79±1.41^{c}	19.00±2.95^{b}
	V2	66.04±0.94^{a}	2.97±0.13^{c}	4.12±0.47^{a}	1.49±0.12^{c}	7.61±1.41^{d}	27.78±1.19^{c}

Values are presented as mean ± SD. Means followed by the same letters in a column are not significantly different at P<0.05 level by the Tukey post hoc test. V1-Variety1, V2-variety 2

The protein and carbohydrate contents of *S. grandiflora* were significantly higher (P<0.05) among the tested GLVs. More flowering variety (V1) of *S. grandiflora* was higher in the aspect of protein, in contrast to the carbohydrate content. Upon the tested GLVs, *S. grandiflora* stands out to be a better source of protein and carbohydrates [2]. In the aspect of crude fat content, all the tested GLVs showed approximately low-fat contents, tempting to be an inauspicious source for dietary fat supplements [2]. However, the crude fiber contents showed a diverse range between varieties and species proving *C. asiatica* varieties to be more promising sources of dietary fiber.

The results of this study manifest the effect of GLV variety on the variation of nutritional factors. The selected GLV varieties were collected from home gardens and grown under similar growth conditions to keep the environmental factors constant, which could affect the nutrition content ineluctably. However, some factors such as the age of the plant (for *S. grandiflora*) and maturity of plant parts could affect the results.

Previous studies also report [5] that two *Alternanthera sessilis* (Mukunuwenna) selections and three *C. asiatica* selections were found to be promising for their high yield potential or good quality characters out of several *A. sessilis* and *C. asiatica* selections.

Even though the attention to indigenous leafy vegetables exhibited a remarkable increase, the knowledge and awareness of GLV selection in agricultural processes is still inadequate. This leads to the cultivation of low-quality GLV varieties, subsequently leading to poor yield and low income. Thus, proper investigation of quality attributes and the factors affecting their variations can be an empowering approach for the combat against malnutrition as well as the development of agriculture. Ultimately the knowledge from this study can be used for the sustainable application of GLV in the Sri Lankan food industry.

Conclusions and Recommendations

Different varieties of GLVs have remarkable variations in nutritional factors such as protein and fiber contents. *S. grandiflora* variety 1, showed the highest protein and carbohydrate contents from the tested GLVs while *C. asiatica* variety 2 was the highest in crude fiber content. This study emphasizes the importance of a thorough investigation of quality characteristics in GLV selection in agriculture.

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