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RESEARCH

Proximate Composition, Phytochemical Content, and Antioxidant Activity of *Basella alba* L. and *Basella rubra* L. in Sri Lanka

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ABSTRACT

Purpose: *Green leafy vegetables (GLVs) are integral to human diets due to their nutritional value. Among these, Basella spp. is notable for its nutrient density. This study aims to assess and compare Basella alba with Basella rubra for their nutritional and phytochemical composition, and antioxidant properties, lacking in the Sri Lankan context, providing insights to enhance crop utilization in agriculture.*

Research Method: *The total chlorophyll and carotene contents, the total flavonoid and phenolic contents were determined by spectrophotometric methods. The antioxidant potential was assessed using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging and ferric ion-reducing antioxidant power (FRAP) assay. The results were analyzed using the Independent Sample t-Test.*

Findings and Values: *The Independent Samples t-Test highlighted that only carbohydrate contents were statistically significantly different ($P<0.05$) between the two species. The higher carbohydrate content was observed in the B. rubra. The aspects of proximate composition except carbohydrate content showed approximately similar results between the two species. The total chlorophyll, carotene, TFC, TPC, and reducing power and radical scavenging ability were statistically significantly different between the species, showing the highest values in B. alba in each aspect. This study compares and highlights the higher antioxidant activity and potential health benefits of B. alba over B. rubra, with new implications for agriculture.*

Keywords: *Antioxidants, Basella alba, Basella rubra, Phytochemicals.*

INTRODUCTION

Basella spp. L. also referred to as Malabar spinach, Ceylon spinach, or Indian spinach, is a perennial climbing vine celebrated for its extensive nutritional and medicinal properties (Chaurasiya *et al*. 2021). This plant is extensively cultivated in tropical and subtropical areas, including America, India, Africa, New Guinea, and Madagascar (Sagar *et al*. 2022). In Sri Lanka, Malabar spinach is widely cultivated in home gardens and small farms due to its ability to grow in a variety of soil types and resilience to local climatic conditions making it an ideal crop for both subsistence and commercial farming.

As a culinary vegetable, Malabar spinach is a vital component of many traditional diets and healthcare practices (Kumar *et al*. 2015, Okouango *et al*. 2019, Kumari *et al*. 2021). This green leafy vegetable (GLV) is not only enriched with essential nutrients including vitamins, minerals, and dietary fibers but also contains a variety of bioactive phytochemicals, including chlorophyll, flavonoids, saponins, alkaloids, and tannins (Adenegan-Alakinde & Ojo 2019, Akinniyi *et al*. 2019, Okouango *et al*. 2019) which contribute significantly to its therapeutic

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potential, including antimicrobial activity, anti-inflammatory activity, nephroprotective ability, and antioxidant activity (Kumari *et al*. 2021). In particular, its antioxidant activity plays a crucial role in mitigating oxidative stress, causative to non-communicable diseases including cardiovascular diseases, diabetes, and cancers (Das & Biswas 2016).

Malabar spinach is easily identified by its morphological characteristics such as broadly ovate, acute leaves, and black seeds. There are two varieties distinctly identified as the green and red varieties. The green variety, popular as a GLV, has a fleshy stem that is stout at the base and slender in its upper branches while the red variety is long, succulent, slender, and much branched. They are commonly discriminated by the green stem and green petiole in the green variety (*Basella alba* L.) and the red stem and red petiole in the red variety (*Basella rubra* L. var. cordifolia (Lamk) Almeida) (Harold 1963, Deshmukh & Gaikwad 2014, Sutor-Świeży *et al*. 2023). Some studies present the red variety as a separate species under the name *Basella rubra* L. (Ranganatha *et al*. 2023). The phylogenetic study of Gayathree *et al*. (2020) produced evidence to identify *B. alba* and *B. rubra* as two species.

However, studies on the nutritional, phytochemical, and antioxidant properties of these species are lacking in the context of Sri Lanka. This study aims to evaluate and compare *B. alba* and *B. rubra* for their proximate composition, and phytochemical contents including total chlorophyll and carotene contents, total flavonoid content (TFC), and total phenolic content (TPC). The antioxidant activity is analyzed via DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity and Ferric ion-reducing antioxidant power. This study can provide significant implications to agriculture and crop science by comparing the popular GLVs with non-popular species, guiding GLV selection for local conditions, leading to sustainable practices and improved resilience, and aiding in establishing breeding programs for enhanced nutrients and phytochemicals. Furthermore, this knowledge can be exploited in the enhancement of agribusiness by product development opportunities and meeting demand for nutritious foods and food security.

MATERIALS AND METHODS

Materials

The large vine-type B. alba and B. rubra (Fig. 1) were collected. Chemical reagents, H_2SO_4, Folin–Ciocalteu reagent, Quercetin, $AlCl_3$, ascorbic acid, Gallic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Na_2CO_3, methanol, and petroleum ether were obtained by Breckland Scientific™, UK. NaOH, $C_6N_6FeK_3$, HgO, K_2SO_4, $Na_2S_2O_3$, CH_3CO_2K, and Zn granules were obtained from Merck, Germany.

Sample preparation

B. alba and *B. rubra* were taxonomically identified according to the morphological characteristics reported by Harold (1963) and Deshmukh & Gaikwad (2014). They were grown in pots under similar growth conditions (soil, water, humidity, sunlight, etc.) in *Gampaha*, Sri Lanka (GPS coordinates - 6.9754° N, 79.9156° E). The edible parts; leaves and young stems were properly cleaned using tap water and distilled water to remove of dust, mud, and other possible impurities. They were air dried at room temperature (25 °C) under shade for one hour, to remove excess water. Then, they were oven-dried (Biotechnologies INC.) at 45 °C until getting a constant weight and finely milled into a powder, using a mortar and pestle. The powdered samples were stored under 4 °C for subsequent chemical analysis.

Sample extraction

For the determination of phytochemical content and antioxidant activity, methanolic extracts were prepared using methanol, as described below. An amount of 20 mg of each *B. alba* and *B. rubra* sample was weighed and mixed by a vortex mixer (BIOLOGIX) with 1 mL of 96% methanol for 1 min. The mixture was centrifuged at 245 g using the high-speed refrigerated micro centrifuge (MX-207, Japan) for 10 min and the supernatant was filtered using a filter paper (Whatman No. 42). The filtrate was stored under 4 °C until analysis.

Figure 1. Photographs showing the morphology of the *Basella* species. A - *Basella alba* L., characterized by green stems and leaves. B - *Basella rubra* L., identified by the reddish-purple stems and leaves.

Moisture content

Moisture content was assessed using the loss on drying approved by the AOAC methods (2002). 2 g of the sample was oven-dried (Biotechnologies INC.) at 95 – 100 °C until they get a constant weight. The moisture content was figured using the equation given below (Wd: weight loss on drying, Ws: weight of the sample).

$$\text{Moisture Content } (\%) = \frac{W_d \times 100}{W_s} \quad (1)$$

Ash content

Ash content was assessed using the dry ashing method by AOAC methods (2002). 3 g of the sample was ashed for 2 hours using the muffle furnace (BIOBASE) at 600 °C. The ash content was calculated using the following equation (Wa: weight loss on ashing, Ws: weight of the sample).

$$\text{Ash Content } (\%) = \frac{(W_s - W_a) \times 100}{W_s} \quad (2)$$

Crude protein content

Protein content was assessed using the Kjeldahl method by the AOAC method (2002). 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 (2 g of $Na_2S_2O_3$ 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, 6.25 – nitrogen to protein conversion factor (Colonna *et al*. 2016)].

$$\text{Crude Protein } (\%) = \frac{1}{W}\Big[(V_{H_2SO_4} \times 2 \times M_{H_2SO_4}) - (V_{NaOH} \times M_{NaOH})\Big] \times 1.4007 \times 100 \times 6.25 \quad (3)$$

Crude fat content

Fat content was assessed using the Soxhlet extraction method approved by the AOAC (2002) method. 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 removed using rotary evaporation (PHOENIX). The flask was placed in an 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 figured using the given equation (Wa: weight of the flask with fat, Wb: weight of the flask without fat, W: weight of the sample).

$$\text{Crude Fat } (\%) = \frac{(W_a - W_b) \times 100}{W} \quad (4)$$

Carbohydrate content

Carbohydrate content was calculated (Arasaretnam *et al*. 2018) by subtracting the total percentages of ash, protein, fat, and moisture from 100.

$$\text{Carbohydrate } (\%) = 100 - (\text{Ash} + \text{Protein} + \text{Fat} + \text{Moisture}) \quad (5)$$

Crude fiber content

The crude fiber content was assessed using the acid and base digestion method (Lesten & Kingsley 2020). 1.0 g of the homogenous sample powder was boiled in H_2SO_4 (0.128 M) 100 mL for 30 min. The residue was filtered through a muslin cloth and washed well using hot distilled water. Then the residue was boiled for 30 min in NaOH (0.125 M), filtered through muslin cloth, and washed well using hot distilled water. The residue was further washed with acetone and dried in an oven at 105 °C. After drying to a constant weight the residue was weighed and ashed for 2 hours using the muffle furnace at 500 °C. Then the ash content was weighed. The crude fiber content was figured using the equation given below (W_f: weight of fiber, W_a: weight of ash, W: weight of the sample).

$$\text{Crude Fiber } (\%) = \frac{(W_f - W_a) \times 100}{W} \quad (6)$$

Total chlorophyll and total carotene contents

The chlorophyll content and total carotene content were analyzed using the method reported by Gunathilake & Ranaweera (2016). The freshly prepared extract was checked for absorbance at 470, 645, and 662 nm on a spectrophotometer (Thermo Scientific, Finland). Total chlorophyll and total carotene contents were determined using the formulas (Lichtenthaler & Wellburn, 1983). The concentration of each pigment was reported as microgram per gram dry weight (μg /g dw) of the leaf sample.

Total flavonoid content (TFC)

The TFC was assessed by the aluminium chloride colorimetric method (Hossain *et al*. 2017). 2 mL of methanolic extract was added to 0.1 mL of 10% $AlCl_3$ with 0.1 mL of 1 M CH_3CO_2K, and 2.8 mL distilled water. Then incubated for 30 min at room temperature. The absorbance (at 415 nm) was measured using a spectrophotometer (Thermo Scientific, Finland). Quercetin was used as the standard, and the results were reported as Quercetin equivalents (mg QE/g dw) of the samples.

Total phenolic content (TPC)

TPC was assessed using the Folin–Ciocalteu assay of Singleton *et al*. (1999) with some modifications, as reported by Gunathilake *et al*. (2014). 0.5 mL aliquot of the sample extract was added to a 0.1 mL of 0.5 N Folin–Ciocalteu reagent. An incubation of 15 min was carried out at room temperature in a dark place. Then 7.5% Na_2CO_3 2.5 mL was added to the mixture and incubated in the dark for another 2 hours at room temperature. Then the absorbance (at 760 nm) was measured using a spectrophotometer (Thermo Scientific, Finland). The total phenolic concentration was reported as gallic acid equivalents (mg GAE/g dw) of the sample.

DPPH radical scavenging assay

The radical scavenging capacity of the sample extract towards the free radical DPPH was assessed using to the method reported by Hatano *et al*. (1988) with several modifications. Freshly prepared 100 µL of the sample extract was added to 3.9 mL of DPPH (1 mM, 0.5 mL) methanolic solution (freshly prepared) and mixed well using a vortex for 15 seconds. A 30 min incubation was carried out in the dark at room temperature. Then absorbance was measured (517 nm, spectrophotometer-Thermo Scientific, Finland). The radical inhibition percentage was calculated using the given formula (A_c – absorbance of the

control, A_s – absorbance of the sample).

$$\text{Inhibition (\%)} = \frac{A_c - A_s}{A_c} \times 100 \qquad (7)$$

Reducing power (FRAP) assay

The reducing power was assessed using the Ferric ion reducing antioxidant power (FRAP) assay method, reported by Oyaizu (1986). Freshly prepared 1 mL of plant extract was added to 2.5 mL of 0.2 M phosphate buffer (pH 6.6) with 2.5 mL of freshly prepared 1% (w/v) $C_6N_6FeK_3$ solution in a test tube. Then a 20 min incubation was carried out in a 50 °C water bath. Then from a 10% (w/v) $C_2HCl_3O_2$ solution, a 2.5 mL volume was added to the mixture, followed by 10 min centrifugation at 352 g. A 2.5 mL was separated from the upper layer and added to a 2.5 mL of distilled water with 0.5 mL of 0.1% (w/v) $FeCl_3$ solution. Using a spectrophotometer (Thermo Scientific, Finland), the absorbance (at 700 nm) was metered. The reducing power was reported as ascorbic acid equivalents (mg AAE/g dw) of the sample.

Statistical analysis

All experiments were conducted in triplicate, with results presented as the mean ± standard deviation (SD). The data from all assessments were analyzed using the Independent Samples t-Test in IBM SPSS Statistics Data Editor, comparing two independent groups; green and red spinach, to assess whether there is statistical evidence to indicate that the population means are significantly different. 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

Proximate composition and fiber content analysis

The analysis of the proximate composition and fiber content of *B. alba* and *B. rubra* provided significant insights into the nutritional profile. The Independent Samples t-Test highlighted that between the two species, only carbohydrate contents were statistically significantly different (P<0.05). The moisture contents obtained for the two species were not significantly different (Table 1) and were comparable to values reported in the literature, such as Kumar *et al*. (2015) (reported moisture content 92.8%). Correspondingly, the ash content, fat, protein, and fiber contents showed similar results between the two species. When comparing the carbohydrate contents, significantly different between the green and red spinach, higher carbohydrate content was observed in the red spinach. Conversely, previous studies revealed relatively high carbohydrate contents in *B. alba* (2.6%) and *B. rubra* (3%) respectively (Kumar *et al*. 2015). The fat content approximated to the results of Kumar *et al*. 2015 while crude protein contents showed relatively higher values in this study. In contrast, the review of Kumar *et al*. (2022), reveals significant differences between every component of the proximate composition of *B. rubra* and *B. alba*.

Phytochemical content and antioxidant activity

The statistical evidence for the significant differences in the results of the tested phytochemical parameters; total chlorophyll, carotene contents, TFC, TPC, and the antioxidant activity among *B. alba* and *B. rubra* were revealed by the Independent Samples t-Test.

Fig. 2 highlights distinct differences in the phytochemical profiles of *B. alba* and *B. rubra*, *B. alba* showing elevated total chlorophyll (15.31±0.80 µg/g dw), total flavonoid (7.57±0.16 mg QE/g dw), and total phenolic (4.94±0.16 mg GAE/g dw) contents, significantly different from *B. rubra*. The carotene contents of *B. alba* (2.04±0.44 µg/g dw) and *B. rubra* (1.38±0.06 µg/g dw) were not statistically different. This analysis suggests that *B. alba* is nutritionally superior to *B. rubra* in terms of its phytochemical composition, particularly, chlorophyll, flavonoids, and phenolics implicating better antioxidant potential. The study of Kumar *et al.* (2015) manifested total chlorophyll and carotene contents to *B. alba* (chlorophyll - 4.6 μg/g, carotene – 1.9 μg/g) and *B. rubra* (chlorophyll – 13.8 μg/g, carotene – 0.5 μg/g), which is approximately similar to the present study. However, the TPC and

Table 1. The proximate composition and fiber content of *Basella* spp.

Basella spp.	Moisture content (%)	Ash content (%)	Carbohydrate content (%)	Crude protein content (%)	Crudefat content(%)	Crude fiber content(%)
B. alba	91.70±0.75	1.87±0.19	0.39±0.04*	5.44±0.61	0.60±0.03	5.24±0.31
B. rubra	91.67±0.50	1.86±0.18	0.69±0.08*	5.15±0.40	0.63±0.03	5.91±0.45

Values are presented as mean ± SD. *Means are significantly different at P<0.05 level by the Independent Samples t-Test.

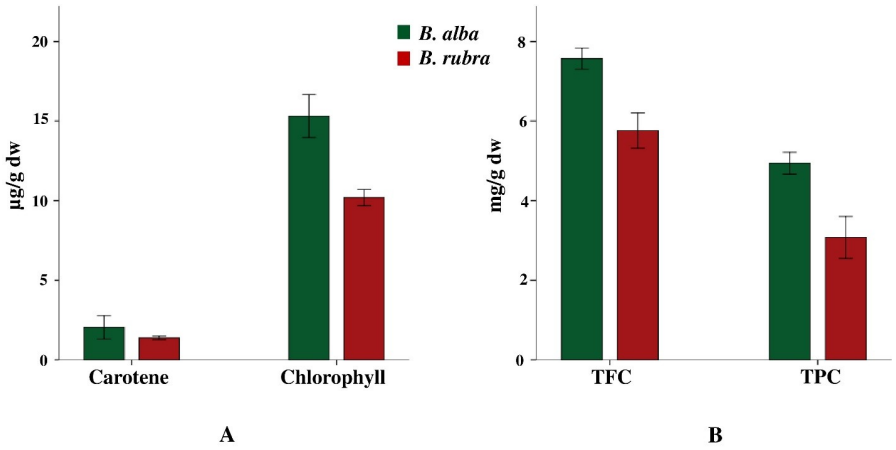

Figure 2. Phytochemical content of *B. alba* and *B. rubra*. A – The total carotene and chlorophyll (μg/g dw) contents. B - The total flavonoid content (TFC) (mg QE/mg dw) and total phenolic content (TPC) (mg GAE/mg dw).

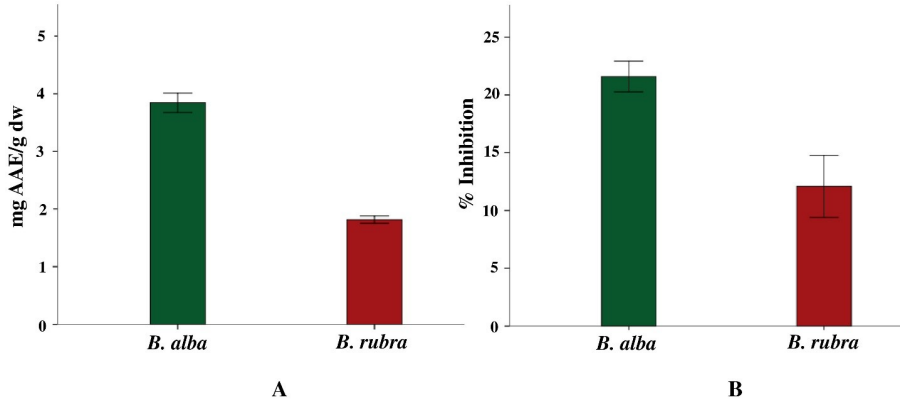

Figure 3. Antioxidant activity of *B. alba* and *B. rubra*. A – The reducing (FRAP) power (mg AAE/g dw). B - The DPPH radical scavenging activity (percentage inhibition).

TFC values reported are remarkably lower in both species when compared with the present study (Kumar *et al.* 2015, Adenegan-Alakinde & Ojo 2019), which indicates possible dissimilarities due to differences in sample preparation, extraction method, analytical techniques, and environmental factors. As displayed in Fig. 3, DPPH radical scavenging activity and reducing power (FRAP) assay indicate significant variations in antioxidant activity between *B. alba* and *B. rubra*. The reducing power (Fig. 3 A), *B. alba* showed a higher reducing power of 3.84±0.10 mg AAE/g dw while *B. rubra* (1.82±0.04 mg AAE/g dw) showed a relatively lower value, which aligns with the results of Jayswal *et al.*

(2021). Despite other experimented aspects, records of the FRAP assay on *B. rubra* is lack in available scientific literature. When considering the DPPH inhibition percentages (Fig. 3 B) were 21.59% in *B. alba* and 12.09% in *B. rubra*, which are higher amounts when compared with the results of previous studies (Adenegan-Alakinde & Ojo 2019). The statistics demonstrate that *B. alba* has a higher antioxidant capacity compared to *B. rubra*, as evidenced by both the FRAP and DPPH assays. This suggests that *B. alba* may provide greater health benefits related to its antioxidant properties. However, the small sample size can reduce the statistical power of the study, make it difficult to detect significant differences, and limit the ability to generalize these findings to broader populations. Environmental factors like temperature, light, and humidity can affect the synthesis of phytochemicals in plants. Therefore, geographical variations affect the nutritional and phytochemical composition, and antioxidant activity of GLVs (Irakli *et al.* 2021, Dahanayaka *et al.* 2025). As this study was conducted under the same environmental conditions, the results might not reflect the natural variability of the *Basella* species. Therefore, future studies could further expand on these findings by including larger sample sizes and broader environmental variation to deepen understanding of phytochemical diversity in *Basella* species.

The findings of this study indicate that green and red spinach have approximate nutritional profiles and distinctly deviating phytochemical and antioxidant profiles, which can influence their selection and use in agricultural and nutritional applications. The higher antioxidant activity observed in *B. alba*, relative to *B. rubra*, indicates meaningful intraspecific variation that can be utilized in future *Basella*-focused breeding or selection programs aimed at improving nutritional quality within this crop. *B. alba* can be cultivated using low-cost cultivation practices under organic farming conditions. By leveraging the health-promoting properties of *Basella alba*, can develop new products and create differentiated offerings in the marketplace, meeting the growing consumer demand for nutritious and functional foods.

CONCLUSIONS

A variation of proximate composition, phytochemical content, and antioxidant activity was observed between the green leafy vegetables *B. alba* and *B. rubra*. Significant differences were observed in chlorophyll, flavonoid, and phenolic contents between *B. alba* and *B. rubra* samples while carotene and proximate composition was approximately similar. The relatively high antioxidant potential of *B. alba* indicates its importance as a functional food. This study provides significant implications to agriculture and crop science by guiding GLV selection, leading to sustainable practices and improved resilience, and aiding in establishing breeding programs for enhanced nutrients and phytochemicals.

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