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Impact of geographical variation on nutritional and antioxidant properties of *Basella alba* L. from Sri Lanka

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Abstract

Background *Basella alba* L. (Malabar spinach) is a widely consumed leafy vegetable, well known for its nutritional and therapeutic properties. These properties arise from the availability of essential nutrients, phytochemicals, and antioxidant potential, which may vary depending on environmental factors induced by the geographical location. In this study our aim is to investigate the correlation between the geographical location and proximate composition, phytochemical content, and antioxidant activity of *B. alba* harvested from fifteen locations in Sri Lanka.

Results According to the statistical analysis by ANOVA and Tukey test, the results of proximate analysis confirmed that samples from different locations showed statistically significant variance in nutritional content. Furthermore, phytochemical content and antioxidant potential varied showing a significant difference between locations in total chlorophyll (27.53 to 6.69 µg/g dry weight), carotene (4.54 to 1.15 µg/g dry weight), total flavonoid content (10.54 to 3.94 mg/g dry weight in Quercetin equivalents), total phenolic content (8.33 to 0.46 mg/g dry weight in gallic acid equivalents), 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (38.03–11.4% inhibition), and ferric ion-reducing antioxidant power (1.23 to 3.76 mg/g dry weight in ascorbic acid equivalents) ($p < 0.05$). The Pearson correlation showed a strong positive correlation between total phenolic content and antioxidant activity. Principal component analysis indicates the role of antioxidant activity and chlorophyll content in location differentiation, forming distinct clusters. Cluster analysis categorized samples into four groups, linking biochemical traits to agro-climatic zones. The principal component analysis and cluster analysis showed a close relationship between some locations due to their high antioxidant and phytochemical accumulation.

Conclusion This study exhibits the importance of geographical location on the phytochemical profile and antioxidant properties of *B. alba*. These findings can be used to refine optimal cultivation sites for *B. alba* to enhance the efficacy of its nutraceutical and pharmaceutical potential.

Keywords Antioxidants, Geographical correlation, Nutritional properties, Phytochemical profile

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Background

Basella alba L., commonly known as Malabar spinach/Ceylon spinach/Indian spinach, is a perennial vine recognized for its diverse nutritional and medicinal properties [1] which is widely cultivated in tropical and subtropical regions [2]. *B. alba* as a culinary vegetable [3] is a vital component of many traditional diets and healthcare practices [3–5]. This green leafy vegetable is not only a rich source of essential nutrients such as vitamins, minerals, and dietary fibers but also contains an array of bioactive phytochemicals, including chlorophyll, flavonoids, saponins, alkaloids, and tannins [5–7]. These compounds contribute significantly to its therapeutic potential including antimicrobial activity, anti-inflammatory activity, nephroprotective effect, and antioxidant activity [3]. Its antioxidant activity plays a crucial role in mitigating oxidative stress which contributes to non-communicable diseases, such as cardiovascular diseases, cancers, and diabetes [8].

B. alba is easily identified by its morphological characteristics such as a fleshy stem that is stout at the base and slender in its upper branches. The leaves are dark green, broadly ovate, 5 to 12 cm in length, and acute, positioned axially. They bear subsessile flowers in white, pink, or red, remaining closed at anthesis. The plant features small, scaly bracts and acute bracteoles. The fruit is black or dark purple, enclosed within a persistent fleshy calyx, and the seeds are black, globose, and indehiscent [9, 10].

The nutritional value, phytochemical composition, and antioxidant activity of *B. alba* can be influenced by various environmental factors such as soil composition, climate, altitude, and agricultural practices, which can differ according to the geographical regions [11, 12]. Understanding these geographical correlations is essential to optimize the cultivation and utilization of *B. alba* for both dietary and medicinal purposes. Previous studies have highlighted that environmental conditions can cause a significant impact on the concentration and efficacy of phytochemicals in plants, affecting their nutritional and antioxidant properties [13, 14]. However, there is a lack of comprehensive research that simultaneously examines the geographical variations in the nutritional profile, phytochemical composition, and antioxidant activity of *B. alba*.

This study aims to address this gap by analyzing the proximate and phytochemical composition along with the antioxidant activity of *B. alba* samples collected from fifteen locations of various Agro-climatic zones which represent different rainfall and altitude levels of Sri Lanka. By establishing these correlations, this research provides valuable insights into how regional conditions influence the nutritional, phytochemical content, and antioxidant activity of *B. alba*. This knowledge can be exploited to guide agricultural practices, improve the quality of herbal medicines, and enhance the nutritional value of this important functional food.

Table 1 Sampling locations of *B. Alba*

Agro-climatic Zone	Sample location	Geographical coordinate	
		Latitude	Longitude
Low-country dry zone (LD)	Jaffna	9.738817	80.01751
	Anuradhapura	8.310921	80.38111
	Polonnaruwa	7.772025	81.211141
	Hambanthota	6.145451	81.144426
	Kalpitiya	8.039823	79.714747
Mid-country intermediate zone (MI)	Badulla	6.907357	81.215564
	Monaragala	6.855969	81.342407
Upcountry intermediate zone (UI)	Ella	6.861539	81.02505
	Welimada	6.869918	80.943397
Low-country wet zone (LW)	Kalutara	6.546935	80.045276
	Hikka	6.130542	80.10267
	Gampaha	7.071295	79.972292
	Rathnapura	6.710008	80.389119
	Kegalle	7.217894	80.2478
Up-country wet zone (UW)	Kandy	7.30824	80.720608

Materials and methods

Materials

Three samples of large vine-type *B. alba* variety with green leaves, white flowers with pink tips and dark purple fruits were collected from each 15 locations in Sri Lanka (Table 1) by following the random sampling method. Each sample was acquired from home gardens, grown with organic farming practices, which included the use of natural compost and manure as fertilizers, avoidance of synthetic pesticides and herbicides, crop rotation and manual weeding. Chemical reagents, H_2SO_4, Folin-Ciocalteu reagent, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Quercetin, $AlCl_3$, ascorbic acid, Gallic acid, Na_2CO_3, methanol, and petroleum ether were obtained by Breckland ScientificTM, 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

The collected samples were taxonomically identified according to the online herbarium, the Atlas of Florida Plants from the Institute for Systematic Botany, the University of South Florida, USA (Accession number – 84672, https://florida.plantatlas.usf.edu/SpecimenDetails.aspx?PlantID=4372). Additionally, the morphological characteristics reported by Deshmukh and Gaikwad, (2014) and Harold, (1963) [9, 10] were referenced. The authenticated samples were deposited in the herbarium of the Department of Plant and Molecular Biology, Faculty of Science, University of Kelaniya, Sri Lanka. Then

they were cleaned well with tap water followed by distilled water to remove dust, mud, and other possible impurities. The edible parts of the samples were air dried at room temperature under shade, to remove excess water. Then dried in a hot air oven (Biotechnologies INC., BTI-HAO-125, India) at 45 °C to obtain a constant weight and ground to fine powder. They were stored at 4 °C for further chemical analysis.

Determination of moisture content

Moisture content was determined and presented on fresh weight basis using the loss on drying by AOAC methods (2002) [15]. 2 g of the sample was dried in a hot air oven (Biotechnologies INC., BTI-HAO-125, India) at 105 °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).

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

Determination of ash content

Ash content was determined and presented on fresh weight basis using the dry ashing method by AOAC methods (2002) [15]. 3 g of the sample was ashed in a muffle furnace (BIOBASE, MC2.5-12, China) at 600 °C for 2 h. The ash content was calculated using the following equation (W_a: weight loss on ashing, W_s: weight of the sample).

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

Determination of crude protein content

Protein content was determined and presented on fresh weight basis using the Kjeldahl method by AOAC method (2002) [15]. 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 [16]].

$$Crude\ Protein\ \% = \frac{\left[\begin{array}{l}(V\ H_2SO_4\ \times\ 2\ \times\ M\ H_2SO_4)\\ -(V\ NaOH\ \times\ M\ NaOH)\end{array}\right] \times\ 1.4007 \times\ 100 \times\ 6.25}{W}$$

Determination of crude fat content

Crude fat content was determined and presented on fresh weight basis using the Soxhlet extraction followed by AOAC method (2002) [15]. 3.0 g of the homogenous sample powder was added to a cellulose thimble and extracted with petroleum ether for 5 h using the Soxhlet apparatus. The solvent was evaporated using a rotary evaporator (PHOENIX, RE 100-S, China). 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).

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

Determination of carbohydrate content

Carbohydrate content was determined [17] by subtracting the sum of the percent of protein, moisture, fat, and ash from 100.

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

Determination of crude fiber content

The crude fiber content was determined and presented on fresh weight basis using the acid and base digestion method [18]. 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 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 h 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).

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

Preparation of methanolic extracts

For the determination of phytochemical content and antioxidant activity, methanolic extracts were prepared

as follows. An amount of 20 mg of each dried sample was weighed and mixed with 1 mL of 96% methanol for 1 min using a vortex. The homogenate was filtered using a Whatman No. 42 filter paper and centrifuged at 245 g using the high-speed refrigerated micro centrifuge (TOMY, MX-207, Japan) for 10 min. The separated supernatant was stored under 4 °C until analysis.

Determination of total chlorophyll and carotene contents

The chlorophyll and carotene contents were analyzed and presented on a fresh weight basis according to the method described by Gunathilake and Ranaweera (2016) [19]. The freshly prepared extract was checked for absorbance at 470, 645, and 662 nm on a microplate photometer (Thermo Scientific™ 51119000, Finland). Total chlorophyll and carotene contents were calculated according to the formulas of Lichtenthaler and Wellburn (1983) (A – absorbance, C_a - Chlorophyll a and C_b - chlorophyll b) [20]. The concentration of each pigment was reported as microgram per gram dry weight (µg /g dw) of the leaf sample.

$$\text{Chlorophyll a} = 11.75\ (\text{A662}) - 2.350\,(\text{A645})$$

$$\text{Chlorophyll b} = 18.61\ (\text{A645}) - 3.960\ (\text{A662})$$

$$\text{Carotene} = 1000\ (\text{A470}) - 2.270\ (C_a) - 81.4\frac{C_b}{227}$$

Determination of total flavonoid content (TFC)

The TFC of the extracts was determined and presented on fresh weight basis by the aluminum chloride colorimetric assay [21]. About 2 mL of extract was mixed with, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M CH_3CO_2K, and 2.8 mL distilled water and incubated at room temperature for 30 min. The absorbance was measured at 415 nm using a microplate photometer (Thermo Scientific™ 51119000, Finland). The TFC was calculated using quercetin as standard, and values were expressed in terms of quercetin equivalents per g dry weight (mg QE/g dw) of the samples (The linear equation for the standard curve; $y = 0.0017x + 0.0679$, $R^2 = 0.9992$).

Determination of total phenolic content (TPC)

TPC was determined and presented on fresh weight basis using the Folin–Ciocalteu assay of Singleton et al., (1999) with some modifications, as described by Gunathilake et al.. (2014) [22, 23]. About 0.5 mL of the sample extract and 0.1 mL of Folin–Ciocalteu reagent (0.5 N) were mixed and incubated at room temperature for 15 min in the dark. Then 2.5 mL 7.5% Na_2CO_3 was added to the mixture and further incubated for 2 h in the dark at room temperature and then the absorbance was measured at 760 nm using a microplate photometer (Thermo Scientific™ 51119000, Finland). The concentration of total phenols was expressed as gallic acid equivalents per g dry weight (mg GAE/g dw) of the sample (The linear equation for the standard curve; $y = 0.0003x + 0.2124$, $R^2 = 0.9922$).

DPPH radical scavenging assay

The scavenging activity of the sample extract towards the free radical DPPH was monitored and presented on fresh weight basis according to the method of Hatano et al.. (1988) with slight modifications [24]. Freshly prepared 100 µL of the extract was dissolved in 3.9 mL freshly prepared methanolic solution of DPPH (1 mM, 0.5 mL). The mixture was vortexed using a vortex mixer (Camlab, MX-S, UK) for 15 s and then left to stand at room temperature for 30 min in the dark. The absorbance was measured at 517 nm using a microplate photometer (Thermo Scientific™ 51119000, Finland). The percentage inhibition of the radicals due to the antioxidant activity was calculated using the following formula (A_s – absorbance of the sample, A_c – absorbance of the control) (The linear equation for the standard curve; $y = -0.0317x + 0.517$, $R^2 = 0.9947$).

$$\%\ \text{inhibition} = \frac{(A_c - A_s)\,100}{A_c} \quad (10)$$

Reducing power (FRAP) assay

The reducing power was determined and presented on fresh weight basis using Ferric reducing antioxidant power (FRAP) assay [25]. Freshly prepared 1 mL of extract was mixed with 2.5 mL of a 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of a 1% (w/v) $C_6N_6FeK_3$ solution. The mixture was incubated in a water bath at 50 °C for 20 min. Then 2.5 mL of 10% (w/v) $C_2HCl_3O_2$ solution was added followed by centrifugation for 10 min at 352 g. A 2.5 mL aliquot of the upper layer was combined with 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) $FeCl_3$ solution. The absorbance was measured at 700 nm using a microplate photometer (Thermo Scientific™ 51119000, Finland). The reducing power was expressed as ascorbic acid equivalents per g dry weight (mg AAE/g dw) of the sample. (The linear equation for the standard curve; $y = 0.0011x + 0.0762$, $R^2 = 0.9968$).

Statistical analysis

All the experiments were designed with three replicates and expressed as mean ± standard deviation (SD). The data for all determinations were subjected to the 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 samples from different locations. Differences were considered

statistically significantly different if the probability values were less than 0.05; the standard alpha level ($p < 0.05$). Pearson's correlation coefficients (r) were then determined between all variables using IBM SPSS Statistics Data Editor. Significant correlations were submitted to principal component analysis (PCA), to observe interrelationships among the samples from different locations. Cluster analysis (CA) was used to identify similarities between the data sets. PCA and CA based on UPGMA were investigated using Past statistics software [26].

Results

Proximate composition analysis

The variation of proximate composition and fiber content of *B. alba* grown in different geographical locations are presented in Table 2. The one-way analysis of variance (ANOVA) test and Tukey test analysis highlighted significant differences ($p < 0.05$) in the nutritional composition of *B. alba* samples obtained from different agro-climatic zones.

According to the results shown in Table 2, the moisture contents of the samples exhibited relatively consistent levels within the same agro-climatic zone. Notably, samples from the low-country wet zone showed the highest moisture content (91.42%). The lowest moisture content level was observed in the dry zone; 80.91%. In ash content, the highest and the lowest values were observed from the locations of the mid-country intermediate zone (MI); Badulla (4.75%) and Anuradhapura (1.42%) from low-country dry zone (LD) respectively. The highest protein content (6.96%) was reported from Welimada (up-country intermediate zone - UI), while Ella from the same agro climatic zone reported the lowest protein content 3.16%. Fat content is relatively consistent across locations, ranging from 0.77 to 0.37% reporting the highest level from Monaragala (MI), while the lowest was from Rathnapura of low country wet zone (LW). The carbohydrate contents showed a remarkable variation between the locations reporting the highest and lowest values from Hambanthota of LD; 11.04% and Kalutara of LW; 0.55%. Fiber content ranges from 6.15% (Monaragala; MI) and 3.30% (Kegalle; LW).

Phytochemical content and antioxidant activity

The results of the one-way analysis of variance (ANOVA) and Tukey test revealed significant differences in the tested phytochemical parameters; total chlorophyll, carotene contents, TPC, and TFC as well as the antioxidant activity among *B. alba* samples based on the geographical origin of the samples.

Figure 1 illustrates the variation of total chlorophyll and total carotene contents depending on the geographical location. The highest chlorophyll content was observed in Ella from the upcountry intermediate zone (27.53 ± 0.94 µg/g dw) (dw - dry weight), while Hikkaduwa from the low-country wet zone (6.69 ± 0.41 µg/g dw) showed the lowest chlorophyll content. The highest carotene content ranged from 4.54 ± 0.03 µg/g dw (Hambanthota from the low-country dry zone) to 1.15 ± 0.28 µg/g dw (Gampaha from low-country wet zone). According to the results, the chlorophyll content varied distinctly comparatively to the carotene content. Furthermore, the carotene content and chlorophyll content showed a contrasting variation across different locations.

Table 2 The proximate composition of *B. Alba* from different locations of five agro-climatic zones in Sri Lanka

Agro-climatic Zone	Location	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Fiber (%)
Low-country dry zone (LD)	Jaffna	86.79 ± 0.97^{bc}	3.83 ± 0.17^{e}	4.42 ± 0.12^{cde}	0.60 ± 0.02^{abcd}	4.36 ± 0.95^{cd}	3.94 ± 0.66^{abc}
	Anuradhapura	84.41 ± 2.14^{ab}	1.42 ± 0.19^{ab}	4.91 ± 0.31^{e}	0.47 ± 0.06^{abc}	9.79 ± 0.72^{ef}	4.08 ± 0.77^{abcd}
	Polonnaruwa	85.15 ± 2.66^{b}	1.66 ± 0.00^{a}	3.23 ± 0.27^{ab}	0.49 ± 0.02^{abc}	10.48 ± 2.42^{f}	3.63 ± 0.54^{ab}
	Hambanthota	80.91 ± 2.73^{a}	3.77 ± 0.38^{e}	3.79 ± 0.41^{abc}	0.49 ± 0.02^{abc}	11.04 ± 3.27^{f}	3.97 ± 0.99^{abc}
	Kalpitiya	86.45 ± 0.64^{bc}	4.08 ± 0.19^{ef}	3.28 ± 0.28^{ab}	0.55 ± 0.07^{abc}	5.65 ± 0.56^{de}	4.32 ± 0.59^{abcde}
Mid-country intermediate zone (MI)	Badulla	89.37 ± 0.32^{cdef}	4.75 ± 0.83^{f}	4.18 ± 0.23^{cde}	0.48 ± 0.05^{cde}	1.22 ± 0.80^{ab}	5.85 ± 0.11^{cde}
	Monaragala	90.04 ± 1.14^{cdef}	3.33 ± 0.33^{de}	4.64 ± 0.21^{de}	0.77 ± 0.08^{de}	1.22 ± 0.86^{abc}	6.15 ± 0.91^{de}
Upcountry intermediate zone (UI)	Ella	87.78 ± 0.47^{bcd}	4.06 ± 0.18^{ef}	3.16 ± 0.25^{a}	0.42 ± 0.08^{a}	4.58 ± 0.78^{bcd}	5.97 ± 0.96^{cde}
	Welimada	88.59 ± 0.06^{cdef}	2.76 ± 0.19^{d}	6.96 ± 0.30^{g}	0.44 ± 0.04^{g}	1.24 ± 0.41^{abc}	4.91 ± 0.94^{abcde}
Low-country wet zone (LW)	Kalutara	91.42 ± 0.35^{ef}	2.55 ± 0.19^{cd}	4.65 ± 0.20^{de}	0.71 ± 0.02^{de}	0.55 ± 0.06^{a}	5.57 ± 0.77^{bcde}
	Hikkaduwa	89.82 ± 0.12^{ef}	2.58 ± 0.10^{cd}	5.96 ± 0.28^{f}	0.42 ± 0.04^{f}	1.22 ± 0.31^{a}	6.13 ± 0.64^{de}
	Gampaha	90.56 ± 0.48^{def}	3.87 ± 0.20^{e}	3.78 ± 0.46^{abc}	0.63 ± 0.14^{abc}	2.29 ± 0.75^{abc}	4.85 ± 0.43^{abcde}
	Rathnapura	89.28 ± 0.25^{cde}	2.86 ± 0.21^{d}	3.99 ± 0.22^{bcd}	0.37 ± 0.07^{bcd}	3.50 ± 0.16^{abcd}	4.07 ± 0.02^{abcd}
	Kegalle	91.18 ± 0.15^{def}	2.87 ± 0.19^{d}	4.01 ± 0.07^{bcd}	0.39 ± 0.02^{bcd}	1.55 ± 0.06^{abc}	3.30 ± 0.54^{a}
Up-country wet zone (UW)	Kandy	89.29 ± 0.57^{cde}	1.75 ± 0.19^{bc}	4.41 ± 0.18^{cde}	0.49 ± 0.08^{cde}	4.07 ± 0.64^{bcd}	4.32 ± 0.55^{abcde}

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

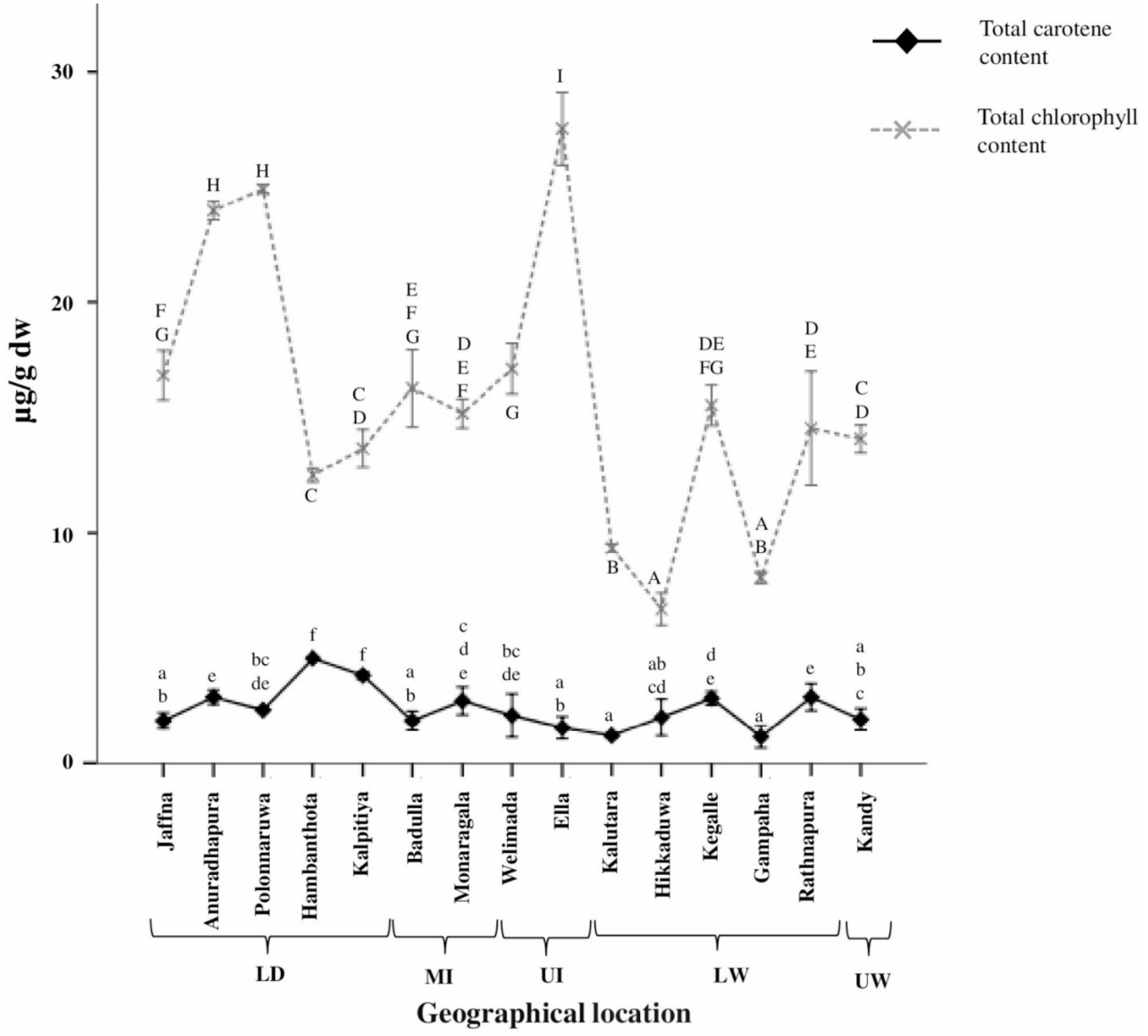

Fig. 1 Variation of total chlorophyll and total carotene contents of *B. alba* with geographical location. The data represent the mean ± standard deviation for each location. Different lowercase letters (a, b, c, etc.) indicate significant differences ($P < 0.05$) in total carotene content between locations, and uppercase letters (A, B, C, etc.) indicate significant differences in total chlorophyll content. (LD – low-country dry zone, MI – mid-country intermediate zone, UI – upcountry intermediate zone, LW – low-country wet zone, UW – upcountry wet zone)

TFC ranged from 10.54 ± 0.40 mg QE/g dw to 3.94 ± 0.42 mg QE/g dw, Kalpitiya reporting the highest value and Gampaha the lowest as presented in Fig. 2. The TFC of the *B. alba* sample from Ella (10.51 ± 0.35 mg QE/g) was remarkably different from the other locations, except Kalpitiya. Similarly, the TPC, also illustrated in Fig. 2, displayed remarkable variations based on location, ranging from 8.33 ± 0.09 mg GAE/g dw (Polonnaruwa) to 0.46 ± 0.12 mg GAE/g dw (Kandy).

As displayed in Fig. 3, DPPH radical scavenging activity and reducing power (FRAP) assay indicate significant variations in antioxidant activity across different locations. The range of DPPH inhibition percentage was from 38.03 to 11.4%. The highest DPPH radical scavenging activity was observed in samples from Ella, indicating strong antioxidant potential. This is followed by samples from Hikkaduwa and Jaffna, which also exhibited relatively high scavenging activity. Conversely, samples from Rathnapura and Kalpitiya showed the lowest DPPH radical scavenging activity, suggesting a lower antioxidant capacity. The reducing power shows a more uniform trend across different geographical locations compared to the DPPH radical scavenging activity. The FRAP values ranged from 1.23 ± 0.08 to 4.33 ± 0.31 mg AAE/g dw, indicating considerable changes in the pattern of antioxidant activity across the locations when compared with DPPH

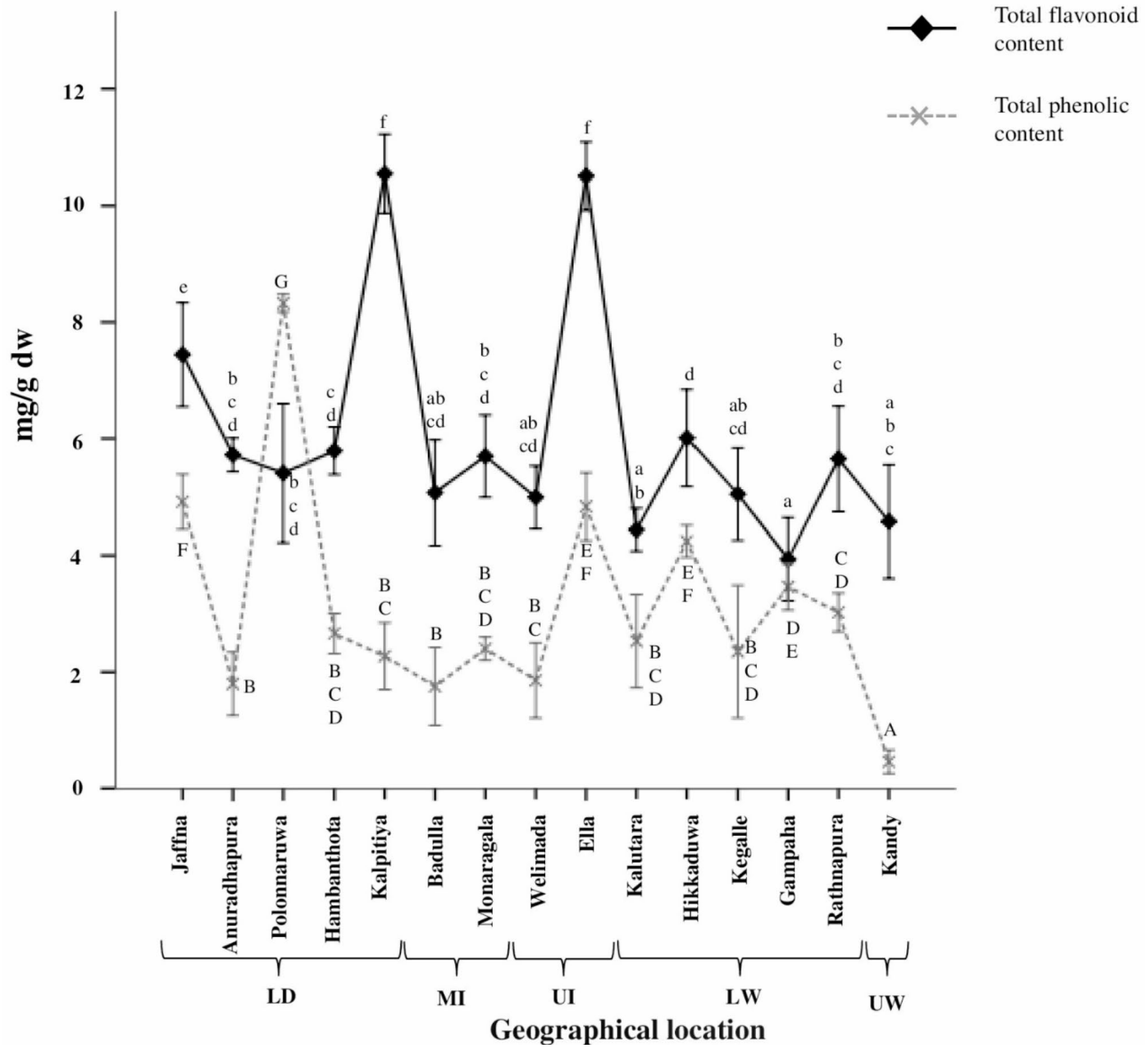

Fig. 2 Variation of TFC and TPC of *B. alba* with geographical location. The data represent the mean ± standard deviation for each location. Different lowercase letters (a, b, c, etc.) indicate significant differences ($P < 0.05$) in total flavonoid content between locations, and uppercase letters (A, B, C, etc.) indicate significant differences in total phenolic content. (LD – low-country dry zone, MI – mid-country intermediate zone, UI – upcountry intermediate zone, LW – low-country wet zone, UW – upcountry wet zone)

radical scavenging activity. The highest reducing power were observed in samples from Polonnaruwa followed by Ella while the lowest was from Rathnapura similarly in DPPH radical scavenging activity.

Correlation study results

The results of the Pearson correlation are presented in Table 3, with an asterisk mark to display the correlations that are statistically significant at a 0.05 level of significance. According to the results, the highest correlation is observed between carbohydrate content and moisture content which is a strong negative (-0.949) correlation. The correlation between most of the nutritional aspects and phytochemical contents/antioxidant activity is not statistically significant, or not moderately/strongly correlated. However, total carotene content and carbohydrate content showed a moderate, positive correlation of 0.571. Total chlorophyll content exhibits significant positive correlations with TFC (0.496), DPPH radical scavenging activity (0.581), and FRAP (0.561). These moderate correlations suggest that *B. alba* samples with higher chlorophyll, also have higher flavonoid content and enhanced antioxidant activity, both in radical scavenging and reducing power. Total carotene content shows a weakly

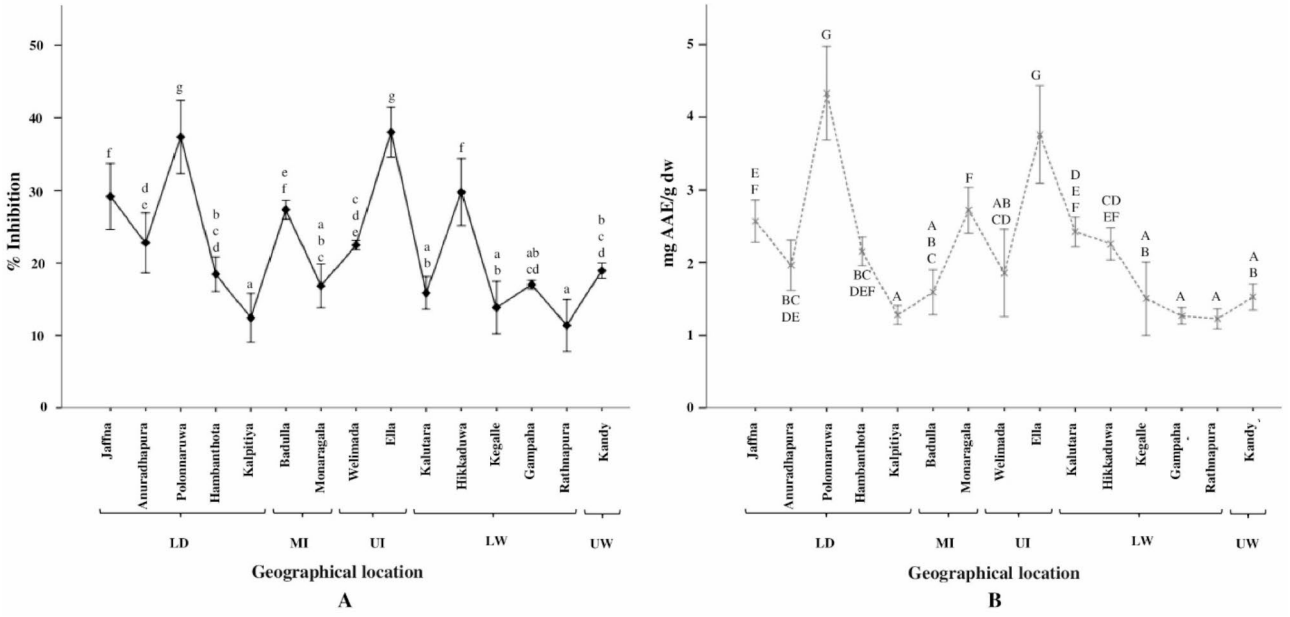

Fig. 3 Variation of antioxidant activity of *B. alba*, employing DPPH radical scavenging percentage (A) and reducing power (FRAP) (B) with geographical location. The data represent the mean ± standard deviation for each location. Different lowercase letters (a, b, c, etc.) indicate significant differences ($P < 0.05$) in DPPH radical scavenging percentage between locations, and uppercase letters (A, B, C, etc.) indicate significant differences in reducing power. (LD – low-country dry zone, MI – mid-country intermediate zone, UI – upcountry intermediate zone, LW – low-country wet zone, UW – upcountry wet zone)

Table 3 Pearson's correlation coefficients between proximate composition and phytochemical content in *B. Alba* samples from 15 geographical locations

	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Fiber (%)	Total chlorophyll content (µg/g dw)	Total carotene content (µg/g dw)	TPC (mg GAE/g dw)	TFC (mg QE/g dw)	DPPH (%)	FRAP (mg AAE/g dw)
Moisture	0.077	0.330*	0.164	-0.949*	0.395*	-0.417*	-0.622*	-0.183	-0.190	-0.263	-0.178
Ash		-0.188	0.121	-0.333*	0.318*	-0.239	0.018	-0.221	0.148	-0.291	-0.126
Protein			-0.017	-0.419*	0.304*	-0.283	-0.194	-0.408*	0.321*	-0.193	-0.041
Fat				-0.209	0.218	-0.252	-0.185	-0.136	-0.072	-0.067	-0.192
Carbohydrate					-0.500*	0.492*	0.571*	0.306*	0.059	0.347*	0.205
Fiber						-0.165	-0.391*	-0.030	-0.108	0.156	0.199
Total chlorophyll content							0.031	0.496*	0.048	0.581*	0.561*
Total carotene content								-0.277	0.335*	-0.178	-0.350*
TPC									-0.216	0.792*	0.750*
TFC										-0.150	-0.093
DPPH											0.746*

*Represents a statistically significant correlation at $p < 0.05$

positive correlation with DPPH radical scavenging activity (0.335) and a negative correlation with FRAP (-0.350). TPC showed a strong correlation with TFC (0.792), DPPH (0.750), and FRAP (0.746). Furthermore, a strong positive correlation was reported between DPPH radical scavenging activity and FRAP (0.746).

A principal component analysis (PCA) was carried out to identify the relationships between various biochemical constituents and antioxidant activities of *B. alba* samples collected from different geographical locations. The variables should be significantly correlated to perform a PCA [27]. Apart from the fat content, all the other nutritional, phytochemical, and antioxidant aspects showed statistically significant correlation coefficients (Table 3). The biplot (Fig. 4) visually represents the first two principal components (PC1 and PC2), which exhibit a significant portion of the total variance in the data set. The tested nutritional, phytochemical, and antioxidant traits were detected in eleven significant components explaining 81.72% of the total variance. In the first component (PC1), 64.3% of the total variance, explains DPPH radical scavenging activity and the total chlorophyll content

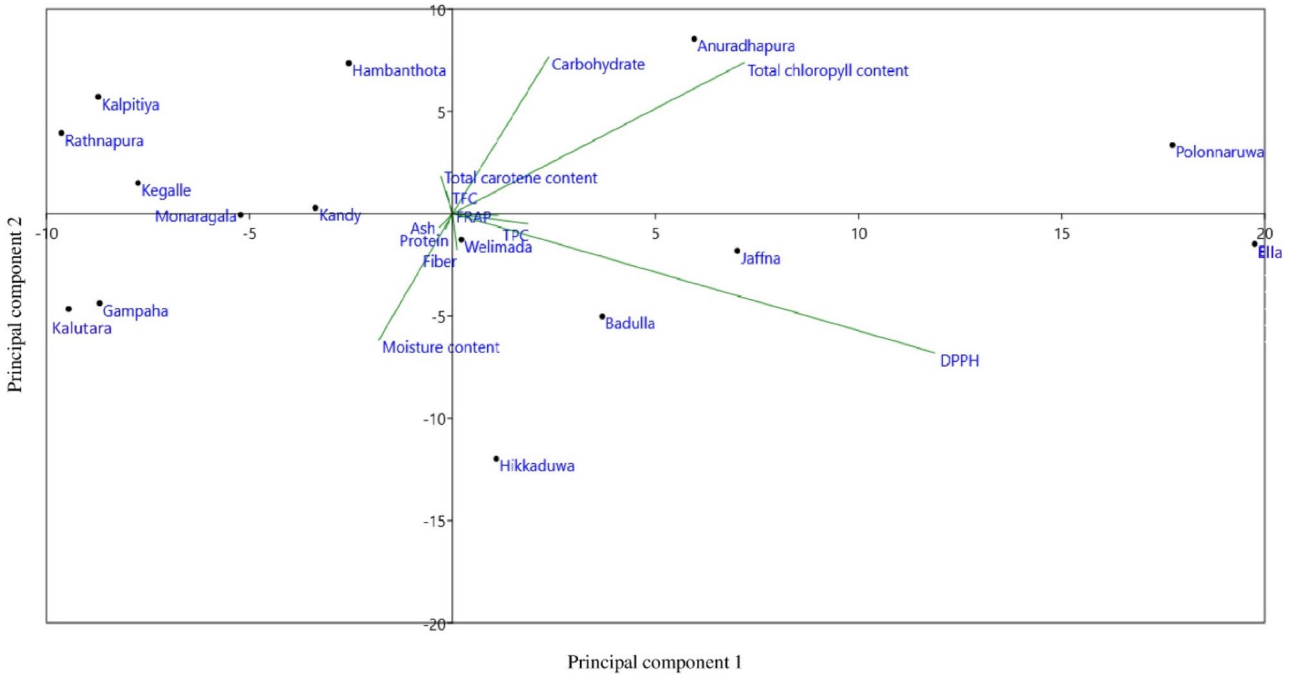

Fig. 4 Biplot of the first two principal components for the investigated geographical locations of *B. alba* samples

of samples had the most portion of the variance. In the second component (PC2), clarifying 20.98% of total variance, carbohydrate and total chlorophyll content of samples had the highest amount of difference of traits (Fig. 4).

DPPH shows a strong positive loading on PC1 (0.82657), indicating that antioxidant activity is a major factor in differentiating the samples. Samples from locations with high antioxidant activity are positioned towards the positive side of PC1. Total chlorophyll content also shows a significant positive loading on PC1 (0.49948), supporting the correlation between chlorophyll content and antioxidant activity. Carbohydrate content shows a strong positive loading on PC2 (0.53349) and a lower positive loading on PC1. Locations with higher carbohydrate content are positioned towards the positive side of PC2.

The PCA biplot also reveals the distinct clustering of samples based on their geographical location. Locations such as Ella and Polonnaruwa, and Jaffna and Anuradhapura form a distinct cluster on the positive side of PC1. Locations such as Anuradhapura and Hambanthota, which have high carbohydrate contents, are positioned on the positive side of PC2. A moisture-rich cluster can be observed from Locations such as Gampaha and Hikkaduwa, which have higher moisture content and are positioned towards the negative side of PC1 and 2.

The UPGMA cluster analysis reveals how the samples group together based on their overall characteristics. The analysis, based on Euclidean distances from the nutritional, phytochemical, and antioxidant traits placed the studied samples from fifteen different locations into four main groups (Fig. 5). The dendrogram reveals four primary clusters (I, II, III, IV) that represent groups of samples with similar biochemical profiles and antioxidant activities. Cluster I, which includes Anuradhapura and Hambanthota from the low-country dry zone, represents the locations with high carbohydrate content and moderate levels of other biochemical constituents and antioxidant activities. Cluster II consists of the samples from Monaragala (MI), Kandy (UW), Rathnapura (LW), Kegalle (LW), Gampaha (LW), Kalutara (LW), and Kalpitiya (LD) which belong to several agro-climatic zones. This cluster includes locations with a diverse range of biochemical properties, indicating varied environmental factors ultimately. The samples in this cluster have moderate levels of protein, fiber, and phenolic content, but lower levels of total chlorophyll and carotene content compared to other clusters. Cluster III includes the locations, Hikkaduwa (LW), Jaffna (LD), Badulla (MI), and Welimada (UI). These locations are characterized by higher moisture content and lower antioxidant activities (DPPH and FRAP) compared to other clusters. Cluster IV consists of Ella and Polonnaruwa with the highest total chlorophyll content and significant antioxidant activities.

Discussion

The present study provides substantial evidence for the high amounts of essential nutrients, phytochemicals, and antioxidant potential in *B. alba* with remarkable

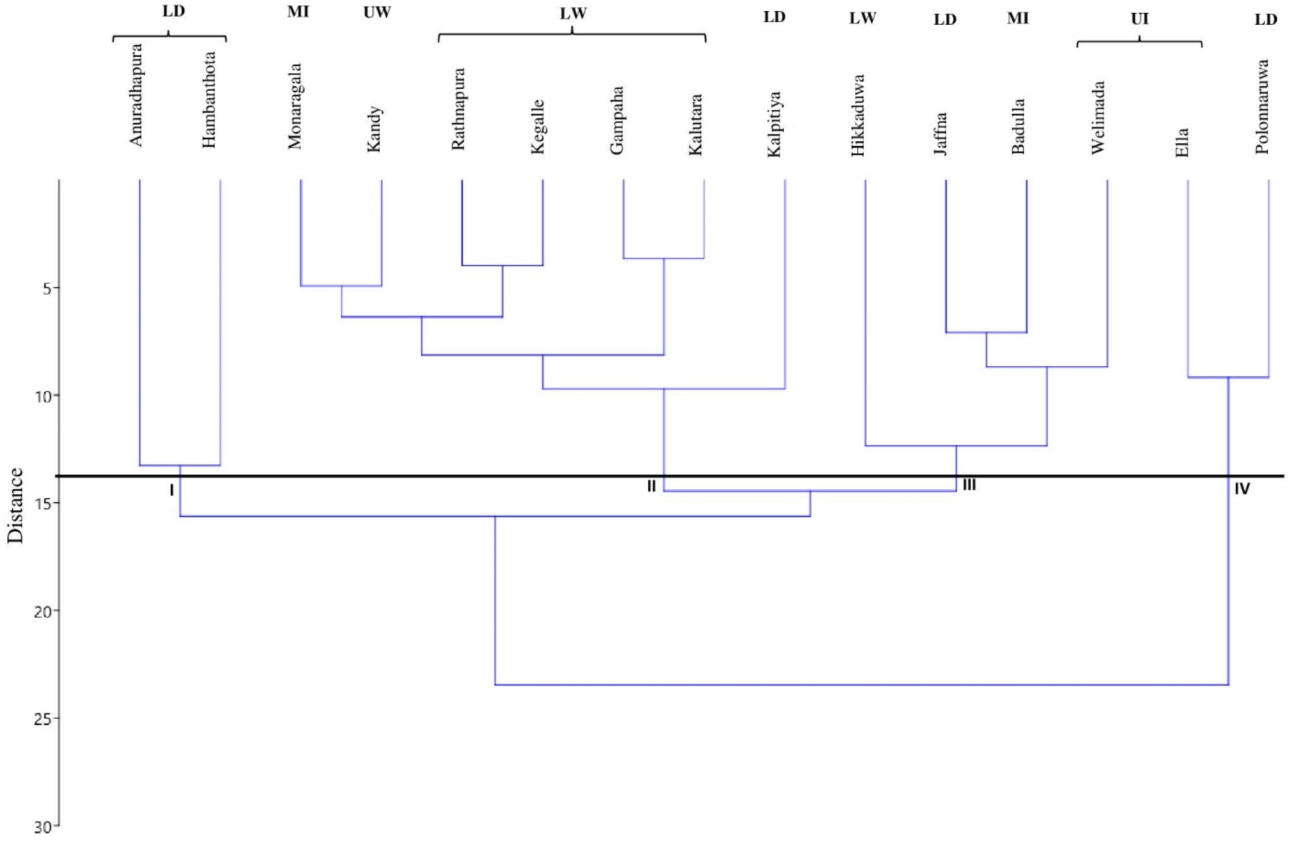

Fig. 5 The UPGMA dendrogram for the nutritional, phytochemical, and antioxidant aspects of *B. alba* samples from different geographical locations using Euclidean distance (LD – low-country dry zone, MI – mid-country intermediate zone, UI – upcountry intermediate zone, LW – low-country wet zone, UW – upcountry wet zone)

variations in each aspect depending on the geographical location of cultivation.

The proximate composition of *B. alba* samples collected from various locations revealed valuable insights about the nutritional profile of the particular green leafy vegetable. According to the results, the highest moisture content (91.42%), approximates the value given by Kumar et al. (2015) (92.8%) [4]. However, a remarkable variation of 91.42–80.91% was observed in the moisture content across different zones suggesting the effect of environmental factors on plant hydration levels. As per the results of this study, the lowest moisture contents are reported from the low country dry zone. Therefore, lower moisture levels might indicate possible drier conditions in the areas while higher moisture levels indicate higher humidity or more consistent water availability in the soil. When comparing with the moisture contents, ash, protein, fat, and fiber contents displayed more distinct variations within agro-climatic zones. According to Tongco et al.. (2015) and Okouango et al. (2019), the ash content of *B. alba* approximates 15% which is a significantly higher value when compared with the results of this study [5, 28] . The ash content in plants represents the total mineral content, which includes both macro and micro-elements and even heavy metals if they are available in the soil [29, 30]. The deviation of ash content from previous studies could have occurred due to differences in mineral availability and heavy metal concentrations in the soil where the plants were grown [31]. The studies of Kumar et al. (2015) and Tongco et al.. (2015) showed significantly different protein contents for *B. alba* which were 1% and 17.55% respectively [4, 28]. The present study's protein content ranged between 6.96% (Welimada) and 3.16% (Ella), which is an average range of results when compared with the results of Kumar et al. (2015) and Tongco et al.. (2015) [4, 28]. The fat contents of the present study which ranged from 0.77 to 0.37% approximates the results of previous studies, 1.1% [4] and 1.58% [28] . The slight variations suggest that fat content in *B. alba* is less influenced by geographical and environmental factors compared to other nutritional components. The total carbohydrate content ranged between 11.04% and 0.55% which is a remarkable inconsistency, particularly in samples from the low-country dry zone, which exhibited significantly higher levels compared to other zones. Previous studies on the total carbohydrate content of *B. alba* showed contrasting results such as 50.62% in Tongco et al.. (2015) while Kumar *et al. (*2015) reported 3% total

carbohydrate content [4, 28]. Previous studies on several plants have reported changes in carbohydrate accumulation according to seasonal changes and environmental factors [32, 33]. Therefore, the deviation of carbohydrate content from previous studies remarks a possible complexity in carbohydrate metabolism in *B. alba* and suggests potential regional variations in carbohydrate accumulation patterns. However, evidence of potential regional variations in carbohydrate accumulation was scares in previous studies on *B. alba*. The crude fiber content which ranged between 6.15% and 3.30% approximates the results of the previous study, 7.23% [28]. The variations of fiber content among locations highlight the influence of local environmental conditions on fiber accumulation in *B. alba*. The locations from Monaragala (MI) and Hikkaduwa (LW) exhibited the highest fiber content, suggesting the possibility of a higher intake of dietary fiber. The lower fiber content could indicate different plant growth dynamics or environmental factors/stressors affecting fiber synthesis.

B. alba is a rich source of phytochemical pigments such as chlorophyll and carotene [5]. The illustrated variations of chlorophyll (Fig. 1) could be influenced by factors such as sunlight exposure, soil fertility, and water availability in these areas. Previous studies revealed that chlorophyll content directly correlates with geographical location which differs from environmental factors such as sunlight [34]. While chlorophyll content shows substantial fluctuation, carotene content remains relatively consistent. This indicates chlorophyll synthesis might be highly sensitive to environmental conditions, nevertheless, carotene synthesis is more stable. When studying the Fig. 1, samples with lower chlorophyll contents tend to show higher carotene contents. Previous studies reported that stress conditions can reduce chlorophyll leading to increased carotenoid levels as a protective mechanism [35, 36]. Notably, the total chlorophyll content shows explicit differences across locations when compared with carotene content. Furthermore, as the highest chlorophyll content (27.53 ± 0.94 µg/g dw) was recorded in Ella (6.861539, 81.02505), situated within the upcountry intermediate zone and, Polonnaruwa and Anuradhapura, both located in the low-country dry zone, reported the second and third-highest values. Conversely, Hikkaduwa which is from the low-country wet zone reported the lowest chlorophyll content (6.69 ± 0.41 µg/g dw). Comparatively low chlorophyll contents were observed in the wet zone samples and higher contents were observed in the dry and intermediate zones. The chlorophyll content is directly correlated with location which differs the environmental factors such as sunlight [34]. Regarding total carotene content, Hamabanthota (6.145451, 81.144426) exhibited the highest concentration (4.54 ± 0.03 µg/g), while Gampaha recorded the lowest (1.15 ± 0.28 µg/g dw). These findings are consistent with those reported by Zhang et al. (2023), showing approximately an average of the above results [37].

The TFC was highest in Kalpitiya (8.039823, 79.714747) (10.54 ± 0.40 mg QE/g dw) and Ella (6.861539, 81.02505) (10.51 ± 0.35 mg QE/g) while, the TPC was highest in Polonnaruwa (7.772025, 81.211141) (8.33 ± 0.09 mg GAE/g dw), suggesting the availability of favorable growth conditions for the biosynthesis of flavonoids and phenolics. Kalpitiya and Polonnaruwa areas belong to the low-country dry zone, which has dry climatic conditions that can induce plant stress responses. Furthermore, high altitude locations such as Ella may expose plants to higher UV radiation, enhancing flavonoid synthesis as a UV-protective mechanism. The TFC in the present study is consistent with the values reported by Aryal et al.. (2019) (6.97 mg QE/g) [38] while the study of Jayswal et al. (2021), *B. alba* TPC (7.79 mg GAE/g dw) approximates the results of the present study [39]. However, according to Sheik et al.. (2023), *B. alba* TPC (57.9 µg GAE/g dw) was significantly lower than the results of the present study which indicates possible dissimilarities due to differences in sample preparation, extraction method, analytical techniques, and environmental factors [40].

The antioxidant potential measured by the DPPH radical scavenging activity showed a significantly different percentage of inhibition between groups (38.03–11.4%) consistent with the results of previous studies [40]. The DPPH radical scavenging activity and the reducing power assay showed higher results in the samples from Ella which is a high-altitude location. Ella location showed the highest TFC and TPC indicating the correlation between phytochemical and antioxidant potential. However, this phenomenon is disrupted in some locations such as Kalpitiya, which is one of the lowest in antioxidant activity, though it has the highest TFC. The divergence between DPPH radical scavenging activity and reducing power across locations highlights the complexity of antioxidant mechanisms in *B. alba*. Furthermore, it suggests that different antioxidant assays could respond differently to environmental factors, and a thorough evaluation of antioxidant activity should be carried out using multiple assays to identify the full antioxidant potential. Furthermore, the significant geographical variation in antioxidant activity shows the importance of selecting optimal cultivation sites to increase the efficiency and effectiveness of the health benefits of *B. alba*. Regions such as Ella, Hikkaduwa, and Jaffna, which showed higher antioxidant activities, could be more suitable for cultivating *B. alba*, especially for nutraceutical and pharmaceutical applications.

The Pearson correlation analysis in this study provides valuable insights into the relationships between nutritional components, phytochemicals, and antioxidant

activities of *Basella alba*. A strong negative correlation between carbohydrate content and moisture content (-0.949) highlights their inverse relationship, a common observation in many plant-based studies. Interestingly, the relationship between nutritional parameters and phytochemical contents or antioxidant activity was generally weak or statistically insignificant. This lack of strong correlations indicates that the nutritional composition of *B. alba* may not directly influence its phytochemical or antioxidant activity. However, a moderate positive correlation between total carotene content and carbohydrate content (0.571) hints at a potential association where increased carbohydrate content might support carotene synthesis or stability. In contrast to nutritional components, phytochemicals exhibited significant correlations with antioxidant activities, underscoring their critical role in determining the functional properties of *B. alba*. According to Sarma and Bhavya (2024), phytochemical contents in green leafy vegetables, often show a stronger correlation with antioxidant activity [41].

Total chlorophyll content showed moderate positive correlations with total flavonoid content (TFC, 0.496), DPPH radical scavenging activity (0.581), and ferric reducing antioxidant power (FRAP, 0.561). These findings suggest that chlorophyll, though primarily associated with photosynthetic activity, might indirectly contribute to antioxidant properties.). Notably, carotene content exhibited a weak positive correlation with DPPH activity (0.335) but a negative correlation with FRAP (-0.350). It suggests that carotene content contributes positively to DPPH activity but might have an inverse relationship with reducing power.

Total phenolic content (TPC) manifested a significant influence in antioxidant activity, exhibiting strong correlations with TFC (0.792), DPPH (0.750), and FRAP (0.746), further indicating that phenolic compounds are closely correlated with flavonoids and antioxidant activity induced by *B. alba*. The study of Gunathilake and Ranaweera (2016) and Neupane and Lamichhane (2020) showed a strong correlation between phytochemical content (TPC and TFC) and antioxidant activity (DPPH and FRAP) in several green leafy vegetables similar to the present study [19, 42]. The results of the correlation study exhibit the importance of phenolics in the antioxidant properties of *B. alba* and they might be participating in a crucial role in radical scavenging and reducing power activities. Furthermore, the strong positive correlation of DPPH radical scavenging activity with FRAP (0.746), suggests that samples with high radical scavenging activity also have a strong reducing power. Amarowicz et al. (2004) and Clarke et al. (2013) reported a similar trend between DPPH radical scavenging activity with FRAP for several plant species [43, 44].

The principal component analysis (PCA) effectively highlights the relationships between biochemical constituents and antioxidant activities of *Basella alba* samples collected from various geographical locations. The first two principal components (PC1 and PC2) collectively explain 81.72% of the total variance, with PC1 accounting for 64.3% and PC2 for 20.98%. This substantial variance indicates that the tested parameters strongly influence the differentiation among the samples. PC1 is primarily influenced by DPPH radical scavenging activity, with a strong positive loading (0.82657), suggesting that antioxidant activity is a major driver of sample variation. Total chlorophyll content also contributes positively to PC1 (0.49948), reinforcing the significant correlation between chlorophyll and antioxidant activity. Geographically, locations such as Ella, Polonnaruwa, Jaffna, and Anuradhapura, which exhibit high DPPH activity and chlorophyll content, cluster together on the positive side of PC1, forming a high antioxidant activity cluster. PC2, explaining 20.98% of the variance, is mainly influenced by carbohydrate content, which shows a strong positive loading (0.53349). Locations such as Anuradhapura and Hambanthota, with elevated carbohydrate levels, are positioned towards the positive side of PC2, forming a high carbohydrate content cluster. Furthermore, these results align with the observed correlations between carbohydrate content and other traits, highlighting its role in differentiating *B. alba* samples according to their location. A distinct moisture-rich cluster is evident on the negative sides of PC1 and PC2, comprising locations such as Gampaha and Hikkaduwa, characterized by high moisture content. This clustering shows the influence of geographical and environmental factors on the biochemical composition of *B. alba*. PCA assumes that the relationships among variables are linear. However, if the actual relationships among nutritional, phytochemical, and antioxidant traits are non-linear, PCA may not accurately capture the underlying patterns in the data. Overall, the PCA biplot provides a comprehensive visual representation of how biochemical and antioxidant traits vary across geographical locations, highlighting the distinct groupings and interrelationships among experimented aspects.

The UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster analysis effectively grouped *Basella alba* samples from fifteen different geographical locations into four distinct clusters based on their nutritional, phytochemical, and antioxidant traits. The clustering patterns reflect the influence of agro-climatic conditions on the biochemical profiles of these samples. Cluster I comprises Anuradhapura and Hambanthota, both located in the low-country dry zone (LD). These samples are characterized by high carbohydrate content and moderate levels of other biochemical and antioxidant

properties. This suggests that the arid conditions in these regions might enhance carbohydrate accumulation while limiting other secondary metabolite synthesis. Cluster II which includes the locations Monaragala (MI), Kandy (UW), Rathnapura (LW), Kegalle (LW), Gampaha (LW), Kalutara (LW), and Kalpitiya (LD), spanning a range of agro-climatic zones, represents a diverse biochemical profile, due to the varied environmental factors influencing plant metabolism in these regions. The samples show moderate levels of protein, fiber, and phenolic content but lower chlorophyll and carotene levels compared to other clusters, suggesting that these traits are less influenced by these regions' environmental conditions. Cluster III comprises Hikkaduwa (LW), Jaffna (LD), Badulla (MI), and Welimada (UI), representing samples with high moisture content but lower antioxidant activity (DPPH and FRAP). The high moisture content might dilute secondary metabolites, explaining the reduced antioxidant activity in these samples. Cluster IV includes Ella and Polonnaruwa, which stand out due to their exceptionally high chlorophyll content and strong antioxidant activities. These locations seem to foster conditions optimal for secondary metabolite accumulation, including phenolics and chlorophyll, which contribute to antioxidant properties. Overall, the UPGMA analysis provides a clear understanding of the biochemical variability among *B. alba* samples, linking their traits to specific agro-climatic zones and highlighting the impact of environmental factors on their functional properties. This analysis assumes that all variables (nutritional, phytochemical, and antioxidant traits) contribute equally to the clustering. However, some traits may have a more significant biological or environmental influence than others, which the method does not account for unless proper investigation of how the contribution is applied.

In this study, a statistically significant difference could be observed in the nutritional content, phytochemical content, and antioxidant activity of *B. alba* samples collected from different geographical locations. The Correlation study showed strong relationships between the biochemical constituents, especially phenolics and flavonoids, and antioxidant activity, as well as the geographical locations. Cluster analysis grouped the samples based on these relationships and provided substantial information about the influence of geographical location on the variations observed. These variations in the nutritional and phytochemical composition of *B. alba* across geographical locations can be attributed to a combination of environmental and methodological factors. Environmental factors induced by the geographical location such as differences in soil composition, pH, temperature, relative humidity, rainfall, sunlight intensity, and UV radiation play a vital role in influencing plant metabolism and, consequently, the biosynthesis of phytochemicals and nutrients. Furthermore, soil fertility and nutrient availability across regions may lead to plant nutrient content variations. Methodological factors, such as the timing of sample collection, post-harvest handling, and the method used to analyze each parameter, could also introduce variability in the measured parameters.

However, this study has some limitations that should be acknowledged. Firstly, the number of sampling sites per location was restricted to one due to the unavailability of *B. alba* samples cultivated under organic farming practices. Though this approach allowed for a uniform comparison, a larger sample size with multiple sample sites could have provided more robust statistical power to detect variations of micro-environmental changes across locations. Also in PCA, the number of geographical locations may be relatively small compared to the number of variables, which could lead to limit the generalizability of the results. Secondly, samples from home gardens grown under organic farming practices might not reflect the properties of *B. alba* grown under conventional farming systems or in the wild. Thirdly, the sampling was conducted within a single season, which does not account for seasonal variations that could influence phytochemical and nutrient content. This study did not incorporate specific environmental factors like soil pH, mineral content, or microclimatic conditions that could directly influence plant properties. Future research should focus on expanding the scope and depth of analysis by increasing the number of sampling sites per location and including more locations across Sri Lanka providing a more comprehensive understanding of the impact of geographical variation on the nutritional and antioxidant properties of *B. alba*. Additionally, future research should incorporate detailed environmental data, such as soil composition, pH, mineral content, and microclimatic conditions, for a more precise assessment of the factors influencing plant properties. By addressing these areas, future studies can build on the findings of this research and contribute to a more holistic approach to the nutritional and therapeutic potential of *B. alba*, across various environmental and agricultural contexts.

Conclusion

A remarkable variation of proximate composition, phytochemical content, and antioxidant activity with the geographical location was observed in *B. alba.* Significant differences in chlorophyll, carotene, flavonoid, and phenolic contents among samples from different locations exhibit the impact of environmental factors on the plant's phytochemical profile. Correlation analysis revealed strong relationships between phytochemicals and antioxidant activities (DPPH and FRAP), indicating its effect on the antioxidant capacity. Principal component analysis and cluster analysis suggested the regions, Ella and

Polonnaruwa for their high-antioxidant potential in *B. alba* and could be optimal cultivation site. The insights of this study can be exploited to guide agricultural strategies such as selecting optimal cultivation sites to increase the nutritional and medicinal value of *B. alba*.

Abbreviations

AAE	Ascorbic acid equivalents
$AlCl_3$	Aluminium chloride
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
$C_6N_6FeK_3$	Potassium ferricyanide
CA	Cluster analysis
CH_3CO_2K	Potassium acetate
DPPH	2,2-Diphenyl-1-picrylhydrazyl
dw	dry weight
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalents
H_2SO_4	Sulfuric acid
HgO	Mercuric oxide
K_2SO_4	Potassium sulphate
LD	Low-country dry zone
LI	Low-country Intermediate zone
LW	Low-country wet zone
m	meter
mm	millimeter
MI	Mid-country intermediate zone
MW	Mid-country wet zone
Na_2CO_3	Sodium carbonate
$Na_2S_2O_3$	Sodium thiosulphate
NaOH	Sodium hydroxide
PC1	Principal component 1
PC2	Principal component 2
PCA	Principal component analysis
QE	Quecertin equivalents
r	Pearson correlation coefficient
SD	Standard deviation
TFC	Total flavonoid content
TPC	Total phenolic content
UI	Upcountry intermediate zone
UK	United Kingdom
UW	Up-country wet zone
Zn	Zinc

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Author contributions
LWD – methodology, data curation, formal analysis, writing ± original draft, writing ± review, and editing; MMSTM – funding acquisition, project administration, conceptualization, supervision, formal analysis, writing ± original draft, writing ± review and editing of the manuscript; CCK – conceptualization, supervision, resources, writing ± review and editing; DU – conceptualization, supervision, writing ± review, and editing. All authors have read and approved the final manuscript.

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Data availability
The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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