Nutritional characters and antioxidant activity of some underexploited minor leafy vegetables of Kerala

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Abstract
The present study investigated the biochemical characters, antioxidant activity, and certain antinutrients of selected minor leafy vegetables *viz*., *Basella alba*, *Cnidoscolus aconitifolius* and *Alternanthera sessilis*, which are grown in Kerala. The results of the study revealed that, *Basella alba* as a notable source of nutrients *viz*., total protein (15.25%), total chlorophyll (136.91mg $100g^{-1}$), and total phenols (152.06mg GAE$100g^{-1}$), along with the lowest antinutrient content including nitrates (0.89mgg^{-1}) and oxalates (13.46mgg^{-1}). *Cnidoscolus aconitifolius* was found to be richest in moisture content (76.20%), crude fibre (31.05%), ascorbic acid content (163.51mg$100g^{-1}$), antioxidant activity (RSA =73.17%), while exhibiting moderate levels of nitrates (0.92mgg^{-1}) and phytates (1.55mgg^{-1}). *Alternanthera sessilis* was reportedly remarkable with the highest total carbohydrate (74.56g$100g^{-1}$), crude fat (3.81%), and total carotenoid content (24.14mg$100g^{-1}$), in addition to moderate oxalate levels (20.43mgg^{-1}), and trace amounts of phytates (0.55mgg^{-1}).

Keywords: *Alternanthera sessilis,* antinutrients*, Basella alba, Cnidoscolus aconitifolius*, radical scavenging activity

Introduction:

Green leafy vegetables (GLVs) have a special place among all vegetables due to their health advantages and abundance in nutrients *viz*., protein, fibre, iron, zinc, folate, β-carotene, and ascorbic acid. India, being blessed with varied climatic conditions, is home to a wide variety of GLVs, some of which are under appreciated but have better nutrient content. Malabar spinach (*Basella alba*), tree spinach (*Cnidoscolus aconitifolius*) and water amaranth (*Alternanthera sessilis*) are among the underutilized leafy vegetables of great attention, due to their adaptability, nutritional composition and health advantages (Table 1), and widely seen in tropical plains of Kerala.

Basella alba is a highly heat-tolerant and rapidly growing perennial vine, widely cultivated as a vegetable during the cooler seasons. It is reported to help in curing various diseases, healing wounds, and androgenic properties (Okouango et al., 2019) since ancient times. *Cnidoscolus aconitifolius* is an attractive shrub which grows 3-5 m tall with broad leaves consisting of 3 or more lobes and fleshy petioles (Breckon, 1979). Kuti and Torres (1996) have proposed that tree spinach leaf tea or decoction might be beneficial in managing the symptoms of

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non-insulin dependent diabetes mellitus (NIDDM). *Alternanthera sessilis* is an annual or perennial prostate weed, grows in the hotter parts of India at an altitude of 1200 m (Jalalpure et al., 2008). *Alternanthera* has been shown to provide various health benefits, including anti-inflammatory properties, cytotoxicity against pancreatic cancer cells, and free radical scavenging activity. In India, it has been used in the treatment of vision and headache, to reduce fever and to treat gastro intestinal problems.

Despite being abundantly accessible in many places of Kerala, these GLVs stand untapped in terms of their nutritional and economic potential. Therefore, a study was made to explore the nutritional characteristics of these minor leafy vegetables, which can be used for value addition of foods, and the results are collectively presented.

Materials and methods

Three minor tropical leafy vegetables selected for the study *viz*., *Basella alba, Cnidoscolus aconitifolius* and *Alternanthera sessilis,* were collected from plains of Thrissur district and grown in the orchard of College of Agriculture, Kerala Agricultural University, Vellanikkara (10.5449° N, 76.2864° E). The fresh apical parts at the vegetative stage of the plants were harvested in the morning, thoroughly cleaned by running tap water, shade dried for one week in ambient conditions until completely dried, and ground to fine powder (Fig.1). The dried powder immediately after drying was collected in clean, dry, air tight plastic containers and subjected to biochemical analyses in the laboratory, Department of Postharvest Management, KAU, Vellanikkara.

A triplicate of dried, powdered leaf samples was used for the analysis. The moisture content was detected using the Association of Official Analytical Chemists' (AOAC, 1980) methods. The crude fibre was estimated by acid alkali method (Chopra and Kanwar, 1978). The crude fat was estimated using the Soxhlet apparatus using petroleum ether as solvent (AOAC, 2012). Total carbohydrates were analyzed using HCl and anthrone reagent. Total phenols were determined by using Folin-Ciocalteau reagent (Sadasivam and Manickam, 1992). The protein concentration was measured following the Lowry et al. method (1951). The ascorbic acid content was estimated using 2, 6 dichlorophenol indophenol dye (AOAC, 1955). Total chlorophyll was estimated according to Ranganna (1977), using 80 per cent acetone. Total carotenoids were determined using 50 mL of ternary solvent (hexane/ethanol/acetone 50/25/25) as followed by Ellong et al. (2015). Antioxidant activity was measured by DPPH (2, 2-diphenyl-1-picryl hydrazyl) radical scavenging assay as suggested by Blois (1958). Marderosian et al. (1980) method was used to assess the oxalate and nitrate content. The phytate was extracted from samples using 2.4% HCl, passing the extract through an AG1-X8 anion-exchange resin to remove inorganic phosphorus, and measuring at 500 nm in spectrophotometer using the Wade reagent (Wheeler and Ferrel, 1971; Latta and Eskin, 1980).

Statistical analysis

The experiments were carried out in triplicates and the data was assessed by one-way ANOVA using software WASP (Web Agri Stat Package) Version 1.0. by ICAR. Values of P±0.05 were considered as statistically significant.

Results and Discussion

Biochemical constituents

The biochemical composition *viz*., moisture content, total carbohydrate, total protein, crude fibre, crude fat, ascorbic acid, total chlorophyll, total carotenoids, and total phenols, of leafy vegetables namely; *Basella alba, Cnidoscolus aconitifolius,* and *Alternanthera sessilis* were estimated and the results are presented in Table 2.

The moisture content significantly varied among selected GLVs, ranging from 68.23 to 76.20 per cent.

The highest value was reported in *Cnidoscolus aconitifolius* (76.20%) and the least in *Alternanthera sessilis* (68.23%), whereas *Basella alba* contained 71.25 per cent moisture. Analogous results were found in the research by Aye (2012), where it was found that Chaya leaves contained notable amount of moisture (72%). According to Badau et al. (2013), increased moisture content enhances the activity of water-soluble enzymes and co-enzymes, which are essential for plant's metabolic processes.

Carbohydrates have a substantial impact on insulin activity. Low glycemic index diets can improve sensitivity to insulin and lower the incidence of type 2 diabetes (Wolever, 2000).Total carbohydrate content values were found to be significantly different among them, with a range of 42.86 to 74.56g100g^{-1}. The highest value is reported in *Alternanthera sessilis* (74.56g100g^{-1}) followed by *Basella alba* (50.27g100g^{-1}), and *Cnidoscolus aconitifolius* (42.86g100g^{-1}). An investigation by Kumar et al. (2016) also revealed that *A. sessilis* leaves are carbohydrate rich when compared to *Celosia argentea* (10.60g100g^{-1}) and *Solanum nigrum* (11.10g100g^{-1}) leaves.

The values of total protein content among the GLVs ranged from 7.74 to 15.25 per cent. *Basella alba* showed significantly highest protein content (15.25%) among the selected GLVs, whereas *Alternanthera sessilis* (7.94%) and *Cnidoscolus aconitifolius* (7.74%) reported almost equivalent values. The findings were higher in *Basella* leaves than in the study conducted by Acho et al. (2015) (9.86%), which would be due to difference in location of study. The protein content in *B. alba* reported in the present study is higher than that of fenugreek (2.26%) which was reported by Yadav (2023). This might be due to different growing conditions and manures applied.

Crude fibre aids in bowel regularity and prevents constipation by adding bulk to the stool (Trowell, 1973), which is abundantly available in GLVs. The results of crude fibre differed significantly among the leafy vegetables, which ranged from 7.24 to 31.05 per cent. *Cnidoscolus aconitifolius* showed the highest crude fibre content with 31.05 per cent followed by *Alternanthera sessilis* (8.75%), and *Basella alba* (7.24%). A study investigated by Orji et al. (2016) revealed almost similar findings (31.16%) in Chaya leaves.

The crude fat present in leafy vegetables offers vital fatty acids and supports the absorption of fat-soluble vitamins (Caunii et al., 2010). Crude fat content was

Table 1. Description of selected underutilized green leafy vegetables

Sl. No.	Botanical name	Common name	Family	Vernacular name	Medicinal properties	References
1	*Basella alba*	Malabar spinach Ceylon spinach Indian spinach Vine spinach	Basellaceae	*Amritvallari* *Malvaa* *Poi* *Potaki* *Upodika* *Vasalacheera* *Vasalakkirai*	Androgenic, anticancer, antiviral, antioxidant, anti-inflammatory, anti-cholesterol, anti-ulcer, antimicrobial, anti-hypoglycemic, and wound healing effect	Kumar et al., 2011; Acikgoz and Adiloglu, 2018; Singh and Sonkar, 2024
2	*Cnidoscolus aconitifolius*	Chayamansa	Euphorbiaceae	*Chaya* *Iyana ipaja* *Pepaya jepang*	Antioxidant, anti-inflammatory, antimicrobial, and cardioprotective	Garcia-Rodriguez et al., 2014; Oluka and Nwankwo, 2023
3	*Alternanthera sessilis*	Water amaranth Sessile joy weed	Amaranthaceae	*Gudari sag* *Honagonesoppu* *Matsyaaksha* *Mukunuwenna* *Ponnakannikeera* *Senchisak*	Treats hazy vision, night blindness, malaria, blood vomiting, and infertility,antibacterial, and anti-cataract	Gunasekera, 2008; Subhashini et al., 2010; Chakraborty and Duary, 2014

Basella alba

Cnidoscolus aconitifolius

Alternanthera sessilis

Figure 1. Selected green leafy vegetables and their leaf powder

identified to be significantly varied among all, ranging from 1.13 to 3.81 per cent. The highest was noticed in *Alternanthera sessilis* (3.81%) and the least in *Cnidoscolus aconitifolius* (1.13%). According to Kumar et al. (2016) *Alternanthera sessilis* leaves contained prominent fat content (2.90%) which is less when compared to the present study. Total fat content reported in commonly known leafy vegetable, i.e., *Murraya koenigii* (2.43%) was also less in comparative study by Parnami and Varma (2019).

Ascorbic acid (vitamin C) is a vital vitamin that is essential to retain good health and protect against multiple illnesses. In addition, collagen production relies on vitamin C content. The findings of ascorbic acid highlight the potential health benefits of the selected GLVs that varied in composition widely between 51.98 and 163.51 mg100g^{-1}. *Cnidoscolus aconitifolius* (163.51 mg100g^{-1}) is found to be richest in vitamin C when compared to *Basella alba* (73.71 mg100g^{-1}) and *Alternanthera sessilis* (51.98 mg100g^{-1}). Ascorbic acid (164.7 mg100g^{-1}) content in *Cnidoscolus aconitifolius* was found higher than spinach (116.39 mg100g^{-1}) in the study conducted by Mugo et al. (2024).

Chlorophyll is a plant pigment which imparts green colour, nutritional significance, and various health advantages by its function in antioxidant characteristics (Korus, 2013). *Basella alba* showed remarkable total chlorophyll content of 136.91 mg100g^{-1} and the values of chlorophyll are found to be different with significant range. *Cnidoscolus aconitifolius* (131.73 mg100g^{-1}) has higher total chlorophyll content when compared to *Alternanthera sessilis* (107.74 mg100g^{-1}). Kumar et al. (2015) also revealed the significant total chlorophyll content in *Basella alba* leaves (138 mg100g^{-1}), which is in line with the present study.

Carotenoids are also plant pigments which are precursors to vitamin A. These versatile isoprenoids exhibit anti-cancerous, anti-diabetic, and antioxidant activity, reducing the risk of chronic vascular diseases (Coyne et al., 2009). In the study, total carotenoids were significantly different among the GLVs, ranging from 10.93 to 24.14 mg100g^{-1}. *Alternanthera sessilis* was significantly prominent in total carotenoid content with 24.14 mg100g^{-1}, while *Basella alba* (10.93 mg100g^{-1}) reported minimal content. *Alternanthera sessilis* (24.14 mg100g^{-1}) possessed highest carotenoid content than *Spinacia oleracea* (9.55 mg100g^{-1}) leaves (Priyadharshana et al., 2022).

Phenolic compounds possess anti-inflammatory, anti-cancerous, and anti-bacterial properties. The

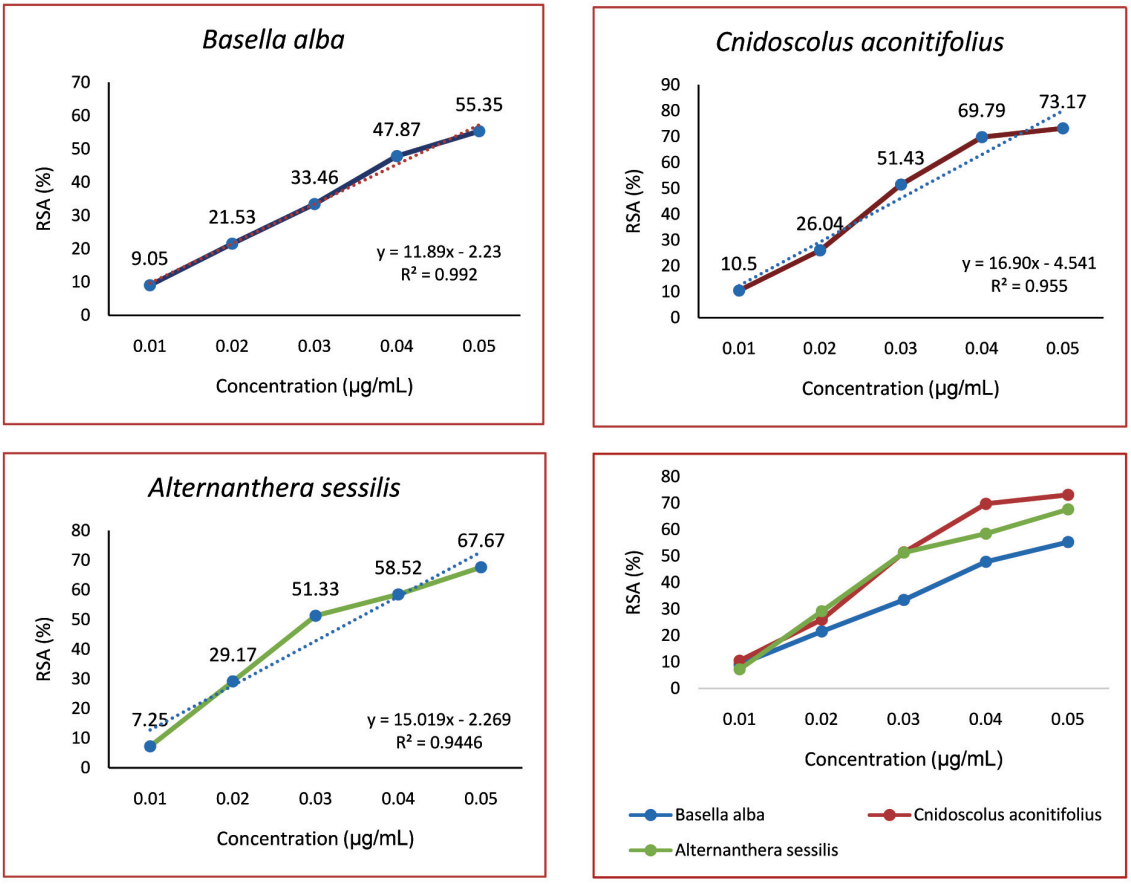

Figure 2. Antioxidant activity of the leafy vegetables (RSA - Radical scavenging activity)

values of total phenols in the study differed significantly from 33.02 to 152.06 mg GAE100g^{-1}. The significantly highest value was shown by *Basella alba* (152.06 mg GAE100g^{-1}), followed by *Alternanthera sessilis* (130.69 mg GAE100g^{-1}), and the lowest in *Cnidoscolus aconitifolius* (33.02 mg GAE100g^{-1}). Comparable results were observed by Kumar et al. (2023) during their research on the retention of bioactive components in *Basella alba* (156 mg GAE100g^{-1}) leaves.

Antioxidant activity

Antioxidant activity (AA) is exhibited by the sample with the highest proportion of radical scavenging activity (RSA). One of the effective, reliable, unique, and reproducible ways to assess the RSA of plant extracts is DPPH assay (Zahid et al., 2017). From the Fig. 2. there was noticeable amount of AA among the selected GLVs with significant difference. *Cnidoscolus aconitifolius* (73.17%) showed the appreciable AA, followed by *Alternanthera sessilis* (67.67%). The AA of *Basella alba* (55.35%) was the minimum. In a similar study investigated by Murugan et al. (2013) *A. sessilis* leaves exhibited the highest RSA at 79.42%, compared to *Amaranthus tristis* (69.45%) which could be due to presence of higher phenolic and flavonoid content.

Antinutritional composition

Antinutrients are natural or synthetic compounds, that inhibit the body's ability to absorb nutrients at their optimal level and interfere with nutrient intake, digestion, absorption, and use (Reddy and Pierson, 1994). They are typically obtained by plants through fertilizers or other natural chemicals. Oxalates,

Table 2. Biochemical profile of the leafy vegetables

Sl. No.	Selected leafy vegetables	Moisture content (%)	Total carbohydrate ($g100g^{-1}$)	Total protein (%)	Crude fibre (%)	Crude fat (%)	Ascorbic acid ($mg100g^{-1}$)	Total chlorophyll ($mg100g^{-1}$)	Total carotenoids ($mg100g^{-1}$)	Total phenols (mg $GAE100g^{-1}$)
1	*Basella alba*	71.25^{b}	50.27^{b}	15.25^{a}	7.24^{c}	1.56^{b}	73.71^{b}	136.91^{a}	10.93^{c}	152.06^{a}
2	*Cnidoscolus aconitifolius*	76.20^{a}	42.86^{c}	7.74^{b}	31.05^{a}	1.13^{c}	163.51^{a}	131.73^{b}	17.99^{b}	33.02^{c}
3	*Alternanthera sessilis*	68.23^{c}	74.56^{a}	7.94^{b}	8.75^{b}	3.81^{a}	51.98^{c}	107.74^{c}	24.14^{a}	130.69^{b}
CD (0.05)		0.780	1.640	0.667	0.147	0.099	1.228	3.838	0.899	2.256
SEM		0.006	0.474	0.193	0.19	0.028	0.355	0.400	0.260	0.652

(Results were expressed as mean of three replications and values followed by different letters are significantly ($P \leq 0.05$) different from each other)

Table 3. Antinutrients of the leafy vegetables

Sl. No.	Selected leafy vegetables	Oxalates (mgg^{-1})	Nitrates (mgg^{-1})	Phytates (mgg^{-1})
1	*Basella alba*	13.46^{c}	0.89^{a}	1.71^{a}
2	*Cnidoscolus aconitifolius*	38.56^{a}	0.92^{a}	1.55^{b}
3	*Alternanthera sessilis*	20.43^{b}	0.97^{a}	0.55^{c}
CD (0.05)		1.676	NS	0.055
SEM		0.484	NS	0.016

(Results were expressed as mean of three replications and values followed by different letters are significantly ($P \leq 0.05$) different from each other)

nitrates, and phytates are major antinutritional factors found in leafy vegetables (Saha et al., 2015). Oxalates affect the absorption and metabolism of calcium and magnesium and causes the risk of developing kidney stones (Holmes et al., 2001).The findings of this study (Table 3.) revealed that, significantly highest oxalate content was present in *Cnidoscolus aconitifolius* (38.56 mgg^{-1}), followed by *Alternanthera sessilis* (20.43 mgg^{-1}), and the least in *Basella alba* (13.46 mgg^{-1}) with significant differing range from 13.46 to 38.56 mgg^{-1}. Lennox and John (2018) found that the total oxalates in Chaya leaves, recorded at 40.30 mgg^{-1}, poses no health risks when consumed.

One of the significant organic substances present in vegetables, nitrate is noteworthy to characterize their overall quality. There was no significant difference in the nitrate content among the selected GLVs. The presence of nitrate was 0.89 mgg^{-1} in *Basella alba*, 0.92 mgg^{-1} in *Cnidoscolus aconitifolius*, and 0.97 mgg^{-1} in *Alternanthera sessilis*. However, the present research demonstrated the least nitrate content when referred to recent observations made by Nnadiukwu and Nnadiukwu (2024) in Chaya leaves (4.61 mgg^{-1}).

Phytic acid functions as the major reservoir for phosphorus in most LVs. In contrast, elevated phytate concentration interferes with zinc homeostasis, chelates mineral cofactors, and negatively affects digestive enzyme activity (Kumari et al., 2004). Phytates were observed higher in *Basella alba* (1.71 mgg^{-1}), followed by *Cnidoscolus aconitifolius* (1.55 mgg^{-1}), and *Alternanthera sessilis* (0.55 mgg^{-1}). Phytates in *Basella alba* (2.2 mgg^{-1}) reported by Choudhury et al. (2017) are higher when compared to the present study.

Conclusion

The study highlights the remarkable potential of the selected green leafy vegetables as valuable food resources. These are the least expensive and rich in total carbohydrates, proteins, crude fibre, antioxidants, ascorbic acid, and other essential nutrients, but their consumption remains limited. In this regard, minor leafy vegetables still need to be researched regarding their nutritional profiles. More systematic studies are encouraged to meticulously register and understand their potential to maximize the industrial use of the specified leafy vegetables.

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