Article published online: 2022-06-27

Nutraceutical Profile of the Ceylon Spinach (*Talinum triangulare*)

Mundamoole Pavithra[1] Kandikere R. Sridhar[1] Ammatanda A. Greeshma[1]

[1] Department of Biosciences, Mangalore University, Mangalagangotri, Mangalore, Karnataka, India

Address for correspondence Kandikere R. Sridhar, Department of Biosciences, Mangalore University, Mangalagangotri, Mangalore, Karnataka, India (e-mail: kandikere@gmail.com).

J Health Allied Sci[NU] 2023;13:38–45.

Abstract

Objective This study appraises the nutraceutical potential of uncooked and cooked Ceylon spinach, the wild leafy vegetable *Talinum triangulare* occurring in southwest India.

Materials and Methods Proximal properties (moisture, crude protein, total lipids, crude fiber, ash, total carbohydrates, and calorific value), mineral contents, amino acid composition, in vitro protein digestibility (IVPD), protein digestibility–corrected amino acid score (PDCAAS), protein efficiency ratios (PERs), and fatty acid methyl esters (FAMEs) were evaluated by following standard protocols.

Results Total lipids, crude fiber, total carbohydrates, and calorific value were higher in cooked than in uncooked samples. Among nine minerals assessed, sodium, magnesium, phosphorous, iron, manganese, and zinc were significantly higher in uncooked samples compared with cooked samples. Lysine, methionine, cystine, tyrosine, alanine, arginine, aspartic acid, glutamic acid, and serine were higher and histidine, isoleucine, leucine, phenylalanine, valine, glycine, and proline were lower in uncooked samples. Cooking improved IVPD, PDCAAS, and PER. Capric and linoleic acids were higher in uncooked samples, while palmitic, 1-pyrrolidinebutanoic, and α-linolenic acid acids were higher in cooked samples. The total unsaturated fatty acids (TUFAs) were higher in cooked samples; however, the total saturated fatty acids (TSFAs) were lower in cooked samples. The ratio of TUFA to TSFA was higher in uncooked than in cooked samples.

Conclusion The protein content of *T. triangulare* is comparable to those of legume seeds and consists of low fat, high fiber, and high carbohydrates along with increased calorific value. The Na/K (<1) and Ca/P (>1) ratios are favorable to combat blood pressure and prevention of calcium loss, respectively. The indispensable amino acids are comparable to or higher than soybean, wheat, and Food and Agriculture Organization of the United Nations and the World Health Organization (FAO-WHO) standards. Improved IVPD, PDCAAS, and PER in cooked samples of *T. triangulare* support its nutraceutical potential in human diet to tackle the protein-energy malnutrition and supplement of its flour strengthens the value of fortified foods. The nutraceutical potential has possible roles to combat lifestyle diseases such as obesity, diabetes, cancers, and cardiac ailments.

Keywords
- proximal qualities
- minerals
- amino acids
- protein bioavailability
- fatty acid methyl esters
- lifestyle diseases
- leafy vegetable

article published online June 27, 2022

DOI https://doi.org/10.1055/s-0042-1746425.
ISSN 2582-4287.

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

Introduction

Leafy vegetables serve as a common human diet worldwide, and they are well known for low fat, low calories, high protein, high dietary fibers, essential minerals, and health-promoting bioactive compounds.[1] Besides their taste and flavor, they are also known as precursors of hormones.[2] Owing to their versatile composition, leafy vegetables are beneficial in the maintenance of health and prevention of many lifestyle diseases.[3] Leafy vegetables could be compared to legumes owing to their capability to supply amino acids.[4–7] Wild leafy vegetables are distributed worldwide, and the Indian subcontinent possesses several such value-added resources used by the local and tribal population. A survey in Hassan District of Karnataka State (India) revealed 45 species of wild leafy vegetables consumed by the rural population as food.[8] Similarly, up to 45 species of underutilized leafy vegetables have been documented from the southern part of Karnataka.[9]

Among the common leafy vegetables, up to 500 species of genus *Talinum* (family: Portulacaceae) are distributed across the world.[10–12] Leaves and tender stems of several species of *Talinum* are well known for their edibility as well as medicinal properties.[12] *Talinum triangulare* is common in southern India, and it grows throughout the year. Besides its nutritional value, *T. triangulare* is also known for its value-added functional attributes and many bioactive compounds, which serve as nutraceutical sources to combat many human ailments (e.g., cardiovascular, diabetes, neurodegeneration).[13–15] The objective of this study is to evaluate the nutraceutical potential of uncooked and cooked *T. triangulare* collected from the southwest coastal region of India.

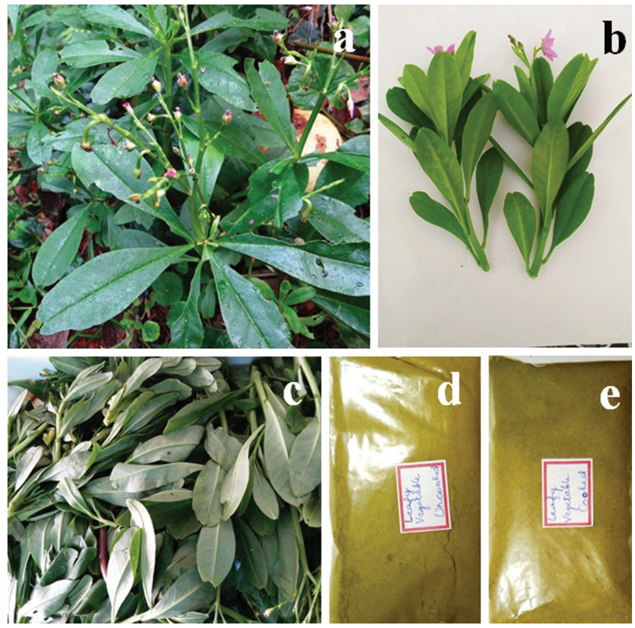

Fig. 1 (**a**) *Talinum triangulare* grown on lateritic soil in southwestern India; (**b**) stem, leaves, and inflorescence; (**c**) harvested tender leaves and stem; (**d**) uncooked dry flour; (**e**) cooked dry flour.

Materials and Methods

Vegetable and Processing

Tender leaves and stem of *T. triangulare* (Jacq.) Willd. (Ceylon spinach or waterleaf) were collected during the rainy season (July 2016) from five locations (~50 m apart) in Payam (Kasaragod District, Kerala;12°29'N, 75°7'E; ►**Fig. 1a, b**). Samples were cleaned in the laboratory by removing inflorescence and basal stem, followed by rinsing in running water to remove debris. Each sample was divided into two parts (►**Fig. 1c**). The first part was dried in an oven (50–55°C) until moisture content drops below 10%, which served as an uncooked sample. Another part was cooked in a household pressure cooker by adding distilled water (3:1) followed by oven drying, which served as a cooked sample. Dried uncooked and cooked samples were powdered and refrigerated (4°C) for analysis (►**Fig. 1d, e**).

Proximal Analysis

The moisture content of leaf flour samples was assessed gravimetrically.[16] Proximal qualities such as crude protein, total lipids, crude fiber, ash, total carbohydrates, and calorific value of samples were evaluated by standard protocols. The crude protein content was assessed by the micro-Kjeldahl method ($N \times 6.25$).[17] The total lipids content was extracted in petroleum ether (60–80°C) using the Soxhlet apparatus according to the Association of Official Agricultural Chemists (AOAC)[16] to evaluate gravimetrically. The crude fiber and ash contents were also determined gravimetrically.[16]

Total carbohydrates were estimated based on the procedure outlined by Sadasivam and Manickam.[18] Samples (100 mg) were taken in boiling tubes and extracted in 2.5N HCl (5 mL) in boiling water bath (3 hours) and cooled to room temperature; then, they were neutralized with solid Na_2CO_3 until effervescence clears. The volume was made up to 10 mL using distilled water and then centrifuged, and the supernatant was analyzed. The test sample (0.2 mL) was made up to 1 mL using distilled water; 5% phenol (1 mL) and 96% H_2SO_4 (5 mL) were added, and the sample was shaken well (10 minutes) and incubated in a water bath (20 minutes); and the absorbance at 490 nm was read using glucose standard to express total carbohydrate in percentage (g/100 g). The calorific value was calculated according to Ekanayake et al.[19]

$$\text{Calorific value (kJ/100 g)} = (\text{crude protein} \times 16.7) + (\text{total lipids} \times 37.7) + (\text{carbohydrates} \times 16.7) \quad (1)$$

Mineral Analysis

The mineral composition was assessed by the protocol proposed by Ramamurthy and Kannan.[20] Moisture-free samples were subjected to field-emission scanning electron microscope–energy dispersive spectrometer (SEM-EDS) analysis (FESEM Carl Zeiss, Oxford Instruments) with a voltage of 15 kV. The SEM images and corresponding EDS spectrum generated for samples were dependent on the properties (shape, shell, and size) to express minerals in percentage. The ratio of Na/K and Ca/P was calculated.

Amino Acid Analysis

Protocols by Hofmann et al[21,22] were employed to analyze the amino acids. The alkali-extracted samples were used for tryptophan analysis and oxidized samples were used for sulfur amino acid analysis. The procedure by Brand et al[23] was followed for the derivatization process of esterification with trifluoroacetylation. Amino acids in reaction vials dried in CH_2Cl_2 served as standards. Samples were hydrolyzed in HCl and evaporated in rotary evaporator (Büchi Laboratorium-stechnik AG RE121) with a diaphragm vacuum pump (MC2C; Vacuubrand GmbH) followed by measurements in GC-C-IRMS/MS. Gas chromatograph (Hewlett-Packard 58590 II) connected through a combustion interface to the IRMS system (GC-C-II to MAT 252, Finnigan MAT) was employed for measurements of GC-C-IRMS/MS for the isotopic determination of nitrogen through a transfer line with a mass spectrometer (GCQ, Finnigan MAT) for determination of the amino acids. The ratio of total essential amino acids (TEAA) to total amino acids (TAA) was calculated (TEAA/TAA).

Protein Quality Assessment

The in vitro protein digestibility (IVPD) was assessed using enzymes (pepsin, trypsin, and α-chymotrypsin) as per the method by Akeson and Stahmann.[24] Samples (100 mg) were treated with pepsin (Sigma, 3,165 units/mg protein; 1.5 mg of pepsin/0.1N HCl [2.5 mL]) at 37°C (3 hours) and inactivated with 1N NaOH (0.25 mL). Again, the samples were incubated by addition of trypsin (Sigma, 16,100 units/mg protein) and α-chymotrypsin (Sigma, 76 units/mg protein; 2 mg each/0.1M potassium phosphate buffer, pH 8.0 [2.5 mL]) at 37°C (24 hours) and further inactivated with 100% trichloroacetic acid (TCA; 0.7 mL). Zero-time control was maintained by inactivation of the enzyme before the addition of samples. The supernatant was collected by centrifugation of inactivated mixture. The residues are washed with 10% TCA (2 mL) and centrifuged. Combined supernatant was pooled twice with diethyl ether (10 mL) and the ether layer was removed by aspiration. Traces of ether in an aqueous layer were eliminated by heating in a boiling water bath (15 minutes). Cooled to room temperature and solution was made up to 25 mL of distilled water. Aliquots (10 mL) were assessed for nitrogen content using the micro-Kjeldahl method[17] to estimate the protein in the digest.

$$\text{IVPD (\%)} = (\text{Protein in digest} \div \text{protein in defatted flour}) \times 100 \quad (2)$$

Essential amino acid score (EAAS) was determined according to the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO-WHO)[25] essential amino acids (EAA(requirement pattern for adults.

$$\text{EAAS} = (\text{Amino acid in the test protein in mg per g}) \div (\text{FAO} - \text{WHO EAA reference pattern in mg per g}) \quad (3)$$

Protein digestibility–corrected amino acid score (PDCAAS) for adult was calculated based on FAO-WHO.[25]

$$\text{PDCAAS} = (\text{EAA in test protein in mg per g}) \div (\text{FAO–WHO EAA reference pattern in mg per g}) \quad (4)$$

Protein efficiency ratio (PER) was determined based on the amino acid composition based on Alsmeyer et al.[26]

$$\text{PER1} = -0.648 + 0.456 \times \text{Leu} - 0.047 \times \text{Pro} \quad (5)$$

$$\text{PER2} = -0.468 + 0.454 \times \text{Leu} - 0.105 \times \text{Tyr} \quad (6)$$

$$\text{PER3} = -1.816 + 0.435 \times \text{Met} + 0.78 \times \text{Leu} + 0.211 \times \text{His} - 0.944 \times \text{Tyr} \quad (7)$$

Fatty Acid Analysis

Total lipids of uncooked and cooked samples extracted by the Soxhlet method were used for the analysis of fatty acid methyl esters (FAMEs).[27] The gas chromatograph (GC-2010, Shimadzu, Japan) combined with an auto-injector (AOI) and capillary column (BPX-70) was used. Elutants were detected on flame ionization detector, and the amplified signals were transferred and monitored using the GC Solutions software (http://www.shimadzu.eu/products/software/labsolutions/gcgcms/default.aspx). Analytical conditions of autosampler, injection port settings, column oven settings, and column information of the gas chromatograph were maintained according to Nareshkumar.[28] The quantity of FAMEs was evaluated based on a comparison of the peaks of spectra and retention time (RT) of peaks, RT and hits of known compounds stocked in the National Institute of Standards and Technology (NIST) library. The ratio of total unsaturated fatty acid (TUFA) to total saturated fatty acid (TSFA) was calculated.

Data Processing

Proximal properties, minerals, amino acids, IVPD, and FAMEs between uncooked and cooked samples were assessed by Student's *t*-test using Statistica version 8.0.[29]

Results and Discussion

Proximal Qualities

Results of seven proximal features have been represented in ►**Table 1**. Moisture content in uncooked and cooked samples was approximately 5% and did not vary significantly ($p > 0.05$). Similar to moisture, crude protein content (30.9–32.8%; $p > 0.05$) was not significantly altered by cooking. The total lipids, crude fiber, total carbohydrates, and calorific value were increased in cooked samples ($p < 0.05$), while the ash content was higher in uncooked samples ($p < 0.01$).

The crude protein content of *T. triangulare* is similar to those in many leafy vegetables as well as legume seeds. Its content in *T. triangulare* (30.9–32.8%) is comparable to those in wild legumes such as *Canavalia cathartica* and *C. maritima* (28–35.5%) occurring in the coastal sand dunes of the southwest coast of India.[30,31] The crude protein content is almost similar to *T. triangulare* grown in Nigeria (30.5%).[1] It did not vary between uncooked and cooked samples (30.9 vs 32.8%; $p > 0.05$) and was comparable with other vegetables such as

Table 1 Proximal characteristics of uncooked and cooked *Talinum triangulare* on a dry weight basis ($n = 5$; mean ± SD)

	Uncooked	Cooked
Moisture (%)	4.83 ± 0.06	5.06 ± 0.1
Crude protein (g/100 g)	30.85 ± 0.12	32.76 ± 1.21
Total lipids (g/100 g)	4.30 ± 0.1	5.64 ± 0.7*
Crude fiber (g/100 g)	14.10 ± 0.15	14.33 ± 0.09*
Ash (g/100 g)	11.88 ± 0.1**	7.08 ± 0.36
Total carbohydrates (g/100 g)	38.17 ± 0.76	40.33 ± 0.58*
Calorific value (kJ/100 g)	1,314.6 ± 16.97	1,433.3 ± 55.31*

Note: Asterisks represent significant differences (*t*-test: $^{*}p < 0.05$; $^{**}p < 0.01$).

Amaranthus hybridus (33%), *A. incurvatus* (31.5%), *Asparagus officinalis* (32.7%), *Brassica oleracea* (34.2%), and *Telfairia occidentalis* (31.2%).[32–35]

The total lipid of *T. triangulare* was higher in cooked samples (4.3 vs 5.6%; $p < 0.05$), which is lower than those in leafy vegetables such as *B. oleracea* (11.9%), *Gnetum africanum* (7.1%), *Moringa oleifera* (9.3%), and *T. occidentalis* (10%),[32,35] while the total lipid in uncooked *T. triangulare* is comparable to that in *Cucurbita pepo* leaves (4.2%).[32] The low lipid content in *T. triangulare* is advantageous in the human diet in combating obesity.

Crude fiber increased in cooked samples (14.1 vs 14.3%; $p < 0.05$). The crude fiber contents in uncooked and cooked samples (14.1 and 14.3%) are high compared with that in *T. triangulare* grown in Nigeria (8.9%).[1] The crude fiber content might have increased owing to loss of minerals, which has been reflected in the significant loss of ash content. Such variations were also seen in the edible fern *Diplazium esculentum*.[36] The fiber content in *T. triangulare* is higher than those in other leafy vegetables such as *Amaranthus viridis* (11.9%), *M. oleifera* (9.4%), and *T. occidentalis* (2.6%),[35,37] while it is comparable to that in *B. oleracea* (14%).[35] The high fiber content in *T. triangulare* is advantageous in the human diet as it helps in the improvement of digestibility (traps fewer proteins and carbohydrates), lowers blood cholesterol, and combats the risks of bowel and colon cancers.[38–40] The high fiber content is also known to delay the conversion of starch into simple sugars, which helps to control diabetes.[41]

Ash was higher in uncooked samples (11.9 vs 7.1%; $p < 0.01$), which is more than that found in *T. triangulare* grown in Nigeria (2.9%).[1] However, the ash content in *T. triangulare* is lower compared with those in other vegetables (e.g., *A. hybridus*, *A. viridis*, *C. pepo*, and *T. occidentalis*).[32,35,37] The low ash content in cooked samples of *T. triangulare* has been reflected in the loss of several minerals (see ►**Table 2**).

The total carbohydrate content in cooked *T. triangulare* increases (38.2 vs 40.3%; $p < 0.05$), but is lower compared with *A. viridis* (49.5%), *Chlorophytum comosum* (65.8%), *M. oleifera* (42.2%), and *T. triangulare* (55.9%).[1,34,35,37] The carbohydrate content in *T. triangulare* was also lower than those of leafy vegetables consumed in southern Côte d'Ivoire (42.9–55.6%),[3] but was higher than other vegetables such as *A. hybridus* (15.4%), *A. officinalis* (34.7%), *B. oleracea* (19%), *C. pepo* (30.4%), *G. africanum* (30.4%), and *T. occidentalis* (35%).[32,34,35] *T. triangulare*, both uncooked and cooked, is endowed with an adequate quantity of carbohydrates as a source of energy and is also capable of combating intestinal cancers and type II diabetes.[42]

Table 2 Mineral composition of uncooked and cooked *Talinum triangulare* (g/100 g) ($n = 5$; mean ± SD)

	Uncooked	Cooked	NRC-NAS–recommended pattern[a]		ICMR-recommended values[b]	
			Children	Adults	Children	Adults
Na	0.05 ± 0.0005*	0.04 ± 0.0006	0.12–0.4	0.5	0.6–1	1.9–2.1
K	13.24 ± 0.1	12.52 ± 0.59	0.5–1.6	1.6–2	1.1–1.6	3.2–3.8
Ca	1.22 ± 0.01	1.15 ± 0.06	0.6–0.8	0.8	–	–
Mg	1.99 ± 0.02*	1.49 ± 0.08	0.06–0.17	0.28–0.35	–	–
P	0.46 ± 0.006*	0.36 ± 0.02	0.5–0.8	0.8	0.6–0.8	0.6–1.2
Fe	0.44 ± 0.006***	BDL	0.01	0.01–0.015	–	–
Mn	0.17 ± 0.002**	0.06 ± 0.006	0.0003–0.003	0.002–0.005	0.004	0.004
Zn	0.05 ± 0.02***	BDL	0.005–0.01	0.012–0.015	–	–
S	0.58 ± 0.03	0.56 ± 0.006	–	–	–	–
Na/K ratio	0.004	0.003	–	–	–	–
Ca/P ratio	2.63	3.20	–	–	–	–

Abbreviations: BDL, below detectable level; ICMR, Indian Council of Medical Research; NRC-NAS, National Research Council-National Academy of Sciences.
Note: Asterisk represent significant differences (*t*-test: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$).
[a]NRC-NAS–recommended pattern.[46]
[b]ICMR-recommended pattern.[47]

Table 3 Amino acid composition of uncooked and cooked *Talinum triangulare* in comparison with soybean, wheat, and FAO-WHO–recommended[25] pattern for adults (g/100 g protein) ($n = 5$, mean ± SD)

	Uncooked	Cooked	Soybean[a]	Wheat[b]	FAO-WHO[c]
Essential amino acid					
Histidine	1.94 ± 0.02	2.06 ± 0.01*	2.50	1.9–2.6	1.90
Isoleucine	5.04 ± 0.006	5.79 ± 0.04**	4.62	3.4–4.1	2.80
Leucine	7.30 ± 0.17	9.96 ± 0.03**	7.72	6.5–7.2	6.60
Lysine	13.04 ± 0.04**	8.87 ± 0.21	6.08	1.8–2.4	5.80
Methionine	2.43 ± 0.01**	1.52 ± 0.02	1.22	0.9–1.5	2.50[d]
Cystine	0.22 ± 0.006*	0.05 ± 0.02	1.70	1.6–2.6	
Phenylalanine	3.77 ± 0.04	6.74 ± 0.05**	4.84	4.5–4.9	6.30[e]
Tyrosine	3.68 ± 0.02**	3.21 ± 0.02	1.24	1.8–3.2	
Threonine	4.33 ± 0.03	4.32 ± 0.006	3.76	2.2–3.0	3.40
Tryptophan	BDL	BDL	3.39	0.7–1.0	1.10
Valine	5.03 ± 0.12	5.9 ± 0.02*	4.59	3.7–4.5	3.50
Nonessential amino acid					
Alanine	7.45 ± 0.02**	6.53 ± 0.06	4.23	2.8–3.0	
Arginine	6.19 ± 0.01**	4.75 ± 0.02	7.13	3.1–3.8	
Aspartic acid	7.54 ± 0.04**	5.43 ± 0.006	11.30	3.7–4.2	
Glutamic acid	9.77 ± 0.06**	6.49 ± 0.006	16.90	35.5–36.9	
Glycine	10.86 ± 0.01	13.35 ± 0.03**	4.01	3.2–3.5	
Proline	5.40 ± 0.006	9.27 ± 0.06**	4.86	11.4–11.7	
Serine	4.57 ± 0.01**	4.20 ± 0.01	5.67	3.7–4.8	
TEAA/TAA ratio	0.47	0.48	–	–	

Abbreviations: BDL, below detectable level; FAO-WHO, Food and Agriculture Organization of the United Nations and the World Health Organization; TAA, total amino acids; TEAA, total essential amino acids.
Note: Asterisks represent significant differences (*t*-test: $^{*}p < 0.01$; $^{**}p < 0.001$).
[a]Bau et al.[51]
[b]USDA.[52]
[c]FAO-WHO pattern.[25]
[d]Methionine + cystine.
[e]Phenylalanine + tyrosine.

The calorific value of *T. triangulare* was higher in cooked than in uncooked samples (1,433 vs 1,315 kJ/100 g; $p < 0.05$). Cooking *T. triangulare* does not lower its calorific value as there is no loss of protein, lipids, and carbohydrates.

The proximate values of uncooked samples of *T. triangulare* found in this study are different from those reported in earlier studies, possibly due to different geographic distribution such as the tropical American region and Nigeria.[43,44]

Mineral Constituents

Among the nine minerals in *T. triangulare*, potassium was the most dominant, followed by magnesium and calcium (►**Table 2**). Sodium, magnesium, phosphorous ($p < 0.01$), and manganese ($p < 0.001$) were higher in uncooked samples than in cooked samples, while potassium, calcium, and sulfur did not vary between uncooked and cooked samples ($p > 0.05$). Iron and zinc were confined to uncooked samples. Levels of potassium, calcium, magnesium, and phosphorus in uncooked and cooked samples and iron and zinc in uncooked samples were higher than those in *T. triangulare* grown in Nigeria.[1] Potassium, calcium, and phosphorus are higher, while the sodium content in cooked samples is comparable with *Talinum portulacifolium*.[11] The potassium content of *T. triangulare* is higher than those in other vegetables such as *Basella alba*, *B. oleracea*, *Colocasia esculenta*, *Corchorus olitorius*, *M. oleifera*, *Solanum melongena*, and *T. occidentalis* as well as in some underutilized leafy vegetables in Assam, India.[3,35,45]

The potassium, calcium, magnesium, iron, manganese, and zinc contents in *T. triangulare* are higher than the National Research Council - National Academy of Sciences (NRC-NAS)–recommended pattern for infants/children and adults,[46] while the sodium and phosphorus contents are lower. On the contrary, potassium and manganese are higher, while phosphorus is comparable to and sodium is lower than the Indian Council of Medical Research (ICMR)-recommended pattern.[47] Low sodium and high potassium in *T. triangulare* lower the Na/K ratio (<1:0.003–0.004), while high calcium and low phosphorus increase the Ca/P ratio (>1:2.6–3.2). The low Na/K ratio (<1) is beneficial in lowering blood pressure as well as hypertension, while the high Ca/P ratio (>1) prevents the

Table 4 In vitro protein digestibility (IVPD) ($n = 5$, mean ± SD), essential amino acid score (EAAS), protein digestibility–corrected amino acid score (PDCAAS), and protein efficiency ratio (PER) of uncooked and cooked *Talinum triangulare*

	Uncooked	Cooked
IVPD (%)	49.66 ± 1.13	55.96 ± 1.06*
EAAS		
Histidine	1.02	1.08
Isoleucine	1.80	2.07
Leucine	1.11	1.51
Lysine	2.25	1.53
Methionine + cysteine	1.06	0.63
Phenylalanine + tyrosine	1.18	1.58
Threonine	1.27	1.27
Valine	1.44	1.69
PDCAAS		
Histidine	50.65	60.44
Isoleucine	89.39	115.84
Leucine	55.12	84.50
Lysine	111.74	85.62
Methionine + cysteine	52.64	35.25
Phenylalanine + tyrosine	58.60	88.42
Threonine	63.07	71.07
Valine	71.51	94.57
PER		
PER1	2.39	3.42
PER2	2.46	3.72
PER3	1.87	4.02

Abbreviations: EAAS, essential amino acid score; IVPD, in vitro protein digestibility; PDCAAS, protein digestibility–corrected amino acid score; PER, protein efficiency ratio.
Note: Asterisk represents significant difference (*t*-test: $^{*}p < 0.01$).

Table 5 Fatty acid methyl esters (FAMEs) of uncooked and cooked *Talinum triangulare* by Soxhlet extraction (g/100 g lipid) ($n = 5$, mean ± SD)

	Uncooked	Cooked
Saturated fatty acid		
Palmitic acid	22.67 ± 0.02	22.78 ± 0.02*
Capric acid	7.77 ± 0.006***	BDL
1-Pyrrolidinebutanoic acid	BDL	4.85 ± 0.006***
Unsaturated fatty acid		
Linoleic acid	14.96 ± 0.006***	BDL
α-Linolenic acid	BDL	34.43 ± 0.02***
TSFA	30.44 ± 0.01**	27.63 ± 0.02
TUFA	14.96 ± 0.006	34.43 ± 0.02**
TUFA/TSFA ratio	0.49	1.25

Abbreviations: BDL, below detectable level; TSFA, total saturated fatty acid; TUFA, total unsaturated fatty acid.
Note: Asterisks represent significant differences (*t*-test: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$).

loss of calcium through urine and restores calcium in the bones.[48,49] As calcium is involved in bone development, it also prevents rickets as well as osteoporosis.[1] A considerable amount of magnesium is known to be effective against coronary heart diseases as well as stroke.

Amino Acid Composition

Lysine and leucine were the dominant essential amino acids, while glycine was the dominant nonessential amino acid in *T. triangulare* (►**Table 3**). Lysine, methionine, tyrosine, alanine, arginine, aspartic acid, glutamic acid, serine ($p < 0.001$), and cystine ($p < 0.01$) were higher in uncooked samples compared with cooked samples. Isoleucine, leucine, phenylalanine, glycine, proline ($p < 0.001$), histidine, and valine ($p < 0.01$) increased in cooked samples. The quantity of isoleucine, leucine, lysine, tyrosine, threonine, valine, alanine, arginine, aspartic acid, glycine, proline, and serine was higher in *T. triangulare* than in the leafy vegetable *A. hybridus*, while it was reverse for histidine, cystine, and glutamic acid.[50] Most of the essential amino acids present in *T. triangulare* surpassed the FAO-WHO–recommended pattern,[25] so also the amino acid composition of soybean and wheat.[51,52] The quantities of some of the amino acids that increased in cooked samples might be due to interconversion, which has been reflected in the increased TEAA/TAA ratio in cooked samples. These results corroborate with the edible fern *Diplazium esculentum*.[36]

Protein Bioavailability

Protein digestibility and bioavailability are the major aspects that help determine protein quality in the foodstuffs. The difference in protein digestibility is due to the nature of proteins, which alter the digestion.[7] The plant protein digestibility will be impaired owing to the presence of antinutritional factors (e.g., polyphenols, phytic acid, trypsin inhibitors).[7,53] The IVPD of *T. triangulare* improved on cooking (49.7 vs 56%; $p < 0.01$), which serves as an important index to follow the protein bioavailability (►**Table 4**). The IVPD in uncooked and cooked samples of *T. triangulare* was similar to that of untreated/unblanched and differently treated (boil blanching, steam blanching, and boiling + sodium bicarbonate blanching) leaves of *Moringa oleifera* (49.6–53.7%).[54] The PDCAASs for histidine, isoleucine, leucine, phenylalanine + tyrosine, threonine, and valine were high in cooked samples, and they were high for lysine and methionine + cystine in uncooked samples. The pattern of PDCAAS in *T. triangulare* indicates its high protein quality, which has been supported by the EAA score. The PER1–3 ranged from 1.9 to 4 in *T. triangulare*, which is favorable, as those possessing PER up to 2 or >2 have been designated as high-quality foodstuffs.[55]

Fatty Acid Composition

The FAMEs of *T. triangulare* are composed of three saturated and two unsaturated fatty acids (►**Table 5**). The uncooked samples were dominated by palmitic acid, while the cooked samples were dominated by palmitic as well as α-linolenic acid. Palmitic acid was higher in cooked samples ($p < 0.05$). The TSFAs were higher in uncooked samples ($p < 0.001$), while the TUFAs were higher in cooked samples ($p < 0.001$). It is likely that some of the saturated fatty acids are converted into unsaturated fatty acids due to cooking. Cooked edible fern *Diplazium esculentum* also showed significant increase in stearic acid.[36] The palmitic acid content is comparable, while the capric, α-linolenic, and linoleic acids are more than those found in *T. triangulare* grown in Andhra Pradesh, India.[56] The palmitic acid is higher, while the linoleic acid is comparable to that found in the leaves of *Portulaca oleracea*.[57] In addition, *T. triangulare* of southern India is also known to possess α-tocopherol (vitamin E).[56] The TUFA/TSFA ratio increased on cooking (0.5 vs 1.3), which is in favorable range to prevent cardiac diseases.[58]

Conclusion

Leafy vegetables have a significant influence on human nutrition and health owing to their value-added nutraceutical potential. The wild leafy vegetable *T. triangulare* is one of such vegetables that possesses protein content equivalent to the legume seeds with low fat, high fiber, high carbohydrate, and high calorific value. Some of the minerals fulfil the requirement of children and adults with favorable sodium/potassium and calcium/phosphorus ratios. The indispensable amino acids are comparable or higher than soybean and wheat. Increased levels of IVPD, PDCAAS, PERs, and TUFAs in cooked samples qualify *T. triangulare* as a suitable human diet to combat protein–energy malnutrition. There are several options to process this leafy vegetable (e.g., blanching, partial boiling, frying, microwave curing) to employ in fortified foods to combat lifestyle diseases without loss of its nutraceutical potential.

Conflict of Interest
None declared.

Acknowledgments
The authors are grateful to Mangalore University and the Department of Biosciences for the facilities to carry out this study. The authors are also thankful to the editor and referees for constructive suggestions to improve the presentation of this manuscript.

References

1 Agunbiade S, Ojezele M, Alao O. Evaluation of the nutritional, phytochemical compositions and likely medicinal benefits of *Vernomia amygdalina, Talinum triangulare* and *Ocimum basilicum* leafy-vegetables. Adv Biol Res (Faisalabad) 2015;9:151–155
2 Ezekwe CI, Chidinma RU, Okechukwu PCU. The effect of methanol extract of *Talinum triangulare* (water leaf) on the hematology and some liver parameters of experimental rats. Glob J Biotechnol Biochem 2013;8:51–60
3 Acho CF, Zoue LT, Akpa EE, Yapo VG, Niamke SL. Leafy vegetables consumed in Southern Côte d'Ivorie: a source of high value nutrients. J Anim Plant Sci 2014;20:3159–3170
4 Aletor O, Oshodi AA, Ipinmoroti K. Chemical composition of common leafy vegetables and functional properties of their leaf protein concentrates. Food Chem 2002;78:63–68
5 Agbede JO, Adegbenro M, Aletor O, Mohammed AA. Evaluation of the nutrition value of *Vernonia amygdalina* leaf protein concentrates for infant weaning foods. Acta Aliment 2007;36:387–393
6 Aletor O. Nutritive and physico-chemical characteristics of some plants and animal-based protein concentrates. Int J Chem Sci 2010;2:155–165
7 Aletor O. Protein quality evaluation and *in vitro* multi-enzyme digestibility of some plant protein isolates and concentrates. Arch Zootech 2012;15:5–16
8 Kumar PGM, Siddamallayya N. Documentation of wild leafy vegetables of Hassan District, Karnataka. Int J Pure App Biosci 2014;2:202–208
9 Gowthami R, Raghavendra KV, Prakash BG, Brunda SM, Niranjana Kumara B. Survey of underutilised leafy vegetables in South Karnataka of India to attain nutritional security. Agric Res Technol 2016;1:01–06
10 Kumar A, Prasad MNV. Propagation of *Talinum cuneifolium* L. (Portulacaceae), an ornamental plant and leafy vegetable, by stem cutting. Floric Ornam Biotechnol 2010;4:68–71
11 Adithya ES, Sasikumar JM, Krishnakumar KA, Lakshmi MS, Christabel HP. *In vitro* antioxidant activity, mineral content and HPLC analysis of *Talinum portulacifolium* (Forssk.) Asch. ex Schweinf. leaf and stem. Int J Pharm Sci 2012;4:423–429
12 Swarna J, Ravindhran R, Lokeswari TS. Characterization of *Talinum triangulare* (Jacq.) Willd. germplasm using molecular descriptors. S Afr J Bot 2015;97:59–68
13 Pavithra M, Sridhar KR, Greeshma AA. Bioactive attributes of traditional leafy vegetable *Talinum triangulare*. In: Watson RR, Zibadi S, eds. Handbook of Nutrition in Heart Health. Wageningen: Wageningen Academic Publishers; 2017:357–372
14 Pavithra M, Sridhar KR, Greeshma AA. Functional attributes of ethnically edible leafy vegetable *Talinum triangulare*. In: Öztürk M, Sridhar KR, Sarwat M, Altay V, Martinez FMH, eds. Ethnic Knowledge on Biodiversity, Nutrition and Health Security. Vol. 2; Palm Bay, FL: Apple Academic Press; 2022 (in press)
15 Sridhar KR, Pavithra M. Bioactive compounds of Ceylon spinach [*Talinum triangulare* (Jacq.) Willd.]. In: Murthy HN, Pack KY, eds. Bioactive Compounds in Underutilized Vegetables and Legumes. Cham: Springer Nature; 2020:151–168
16 AOAC. Official Methods of Analysis. 16th ed. Washington, DC: Association of Official Analytical Chemists; 1995
17 Humphries EC. Mineral composition and ash analysis. In: Peach K, Tracey MV, eds. Modern Methods of Plant Analysis. Vol. 1;Berlin: Springer; 1956:468–502
18 Sadasivam S, Manickam A. Biochemical Methods. New Delhi: New Age International Pvt. Ltd.; 2008
19 Ekanayake S, Jansz ER, Nair BM. Proximate composition, mineral and amino acid content of mature *Canavalia gladiata* seeds. Food Chem 1999;66:115–119
20 Ramamurthy N, Kannan S. SEM-EDS analysis of soil and plant (*Calotropis gigantea* Linn.) collected from an industrial village, Cuddalore Dt, Tamil Nadu, India. Rom J Biophys 2009;19:219–226
21 Hofmann D, Jung K, Bender J, Gehre M, Schüürmann G. Using natural isotope variations of nitrogen in plants an early indicator of air pollution stress. J Mass Spectrom 1997;32:855–863
22 Hofmann D, Gehre M, Jung K. Sample preparation techniques for the determination of natural 15N/14N variations in amino acids by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). Isotopes Environ Health Stud 2003;39 (03):233–244

23 Brand WA, Tegtmeyer AR, Hilkert A. Compound-specific isotope analysis, extending towards 15N/14N and 13C/12C. Org Geochem 1994;21:585–594
24 Akeson WR, Stahmann MA. A pepsin pancreatin digest index of protein quality. J Nutr 1964;83(03):257–261
25 FAO-WHO. Protein Quality Evaluation. Reports of a Joint FAO-WHO Expert Consultation, Food and Agriculture Organization of the United Nations. Food and Nutrition Paper # 51 Rome: FAO; 1991
26 Alsmeyer RH, Cunningham AE, Happich ML. Equations predict PER from amino acid analysis. Food Technol 1974;28:34–38
27 Padua-Resurreccion AB, Banzon JA. Fatty acid composition of the oil from progressively maturing bunches of coconut. Philipp J Coconut Stud 1979;4:1–15
28 Nareshkumar S. Capillary gas chromatography method for fatty acid analysis of coconut oil. J Plant Crops 2007;35:23–27
29 StatSoft. Statistica, Version # 8. Tulsa, OK: StatSoft Inc; 2008
30 Seena S, Sridhar KR, Jung K. Nutritional and antinutritional evaluation of raw and processed seeds of a wild legumes, *Canavalia cathartica* of coastal sand dunes of India. Food Chem 2005; 92:465–472
31 Seena S, Sridhar KR. Nutritional and microbiological features of little known legumes, *Canavalia cathartica* Thouras and *C. maritima* Thouars of the southwest coast of India. Curr Sci 2006; 90:1638–1650
32 Kizito IME, Angela UC. Nutritional composition of some leafy vegetables consumed in Imo State, Nigeria. J Appl Sci Environ Manag 2009;13:35–38
33 Dickson RA, Annan K, Fleischer TC, Amponsah IK, Nsiah K, Oteng JA. Phytochemical investigations and nutritive potential of eight selected plants from Ghana. J Pharm Nutr Sci 2012;2:172–177
34 Aberoumand A. Evaluation of biochemical contents, trace elements, nutritive value and HPTLC profiling in two edible food plants based diets. Nutr Food Sci Res 2014;1:57–61
35 Iyaka YA, Idris S, Alawode RA, Bagudo BU. Nutrient content of selected edible leafy vegetables. Am J Appl Chem 2014;2:42–45
36 Greeshma AA, Sridhar KR, Pavithra M. Nutritional prospects of edible fern of the Western Ghats of India. In: Öztürk M, Hakeem KR, Ashraf M, Ahmed MSA, eds. Global Perspectives on Underutilized Crops. Cham: Springer International Publishing; 2018:151–164
37 Uwah EI, Moses EA, Okokon KR. Proximate, minerals and antinutrients composition of two leafy vegetables commonly consumed in Idu, Uruan, Akwa Ibom State, Nigeria. Int J Curr Res Chem Pharm Sci 2015;2:24–30
38 Balogun AM, Fetuga BL. Chemical composition of some underexploited leguminous crop seeds in Nigeria. J Agric Food Chem 1986;34:189–192
39 Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. N Engl J Med 1995;333(05):276–282
40 Slavin J, Jacobs D, Marquart L. Whole-grain consumption and chronic disease: protective mechanisms. Nutr Cancer 1997;27 (01):14–21
41 Ogbonnaya EC, Chinedum EK. Bioactive constituents and *in vitro* antioxidant capacity of water leaf (*Talinum triangulare*) as affected by domestic cooking. European J Med Plants 2013;3:540–551
42 Venn BJ, Mann JI. Cereal grains, legumes and diabetes. Eur J Clin Nutr 2004;58(11):1443–1461
43 Rana MK, Brar NS. Ceylon spinach. In: Rana MK, (ed. Vegetable Crops Science. 1st ed. Boca Raton, FL: CRC Press; 2017:891–896
44 Bioltif YE. Review on the medicinal potentials of waterleaf (*Talinum triangulare*). Mediterr J Basic Appl Sci 2020;4:1–7
45 Saha J, Biswal AK, Deka SC. Chemical composition of some underutilized green leafy vegetables of Sonitpur District of Assam, India. Int Food Res J 2015;22:1466–1473
46 NRC-NAS. Recommended Dietary Allowances. Washington, DC: National Academy Press; 1989
47 Food Safety and Standards Authority of India. Available at: https://www.fssai.gov.in/upload/advisories/2020/01/5e159e0a809bbLetter_RDA_08_01_2020.pdf
48 Shills MEG, Young VR. Modern nutrition in health and disease. In: Neiman DC, Buthepodorth DE, Nieman CN, eds. Nutrition. Dubuque, IA: Wm C Brown; 1988:276–282
49 Yusuf AA, Mofio BM, Ahmed AB. Proximate and mineral composition of *Tamarindus indica* Linn 1753 seeds. ScientificWorldJournal 2007;2:1–4
50 Akubugwo IE, Obasi NA, Chinyere GC, Ugbogu AE. Nutritional and chemical value of *Amaranthus hybridus* L. leaves from Afikpo, Nigeria. Afr J Biotechnol 2007;6:2833–2839
51 Bau HM, Vallaume CF, Evard F, Quemener B, Nicolas JP, Mejean L. Effect of a solid-state fermentation using *Rhizopus oligosporus* sp. T-3 on elimination of antinutritional substances and modification of biochemical constituents of defatted rapeseed meal. J Sci Food Agric 1994;65:315–322
52 USDA. Nutrient Database for Standard Reference Release 13, Food Group # 20: Cereal Grains and Pasta. Agriculture Handbook # 8–20 Beltsville, MD: USDA Agricultural Research Service; 1999
53 Aletor VA. Cyanide in garri. 2. Assessment of some aspects of the nutrition, biochemistry and haematology of the rats fed garri containing varying residual cyanide levels. Int J Food Sci Nutr 1993;4:289–295
54 Kirana T, Harijono H, Estiasih T, Sriwahyuni E. Effect of blanching treatments against protein content and amino acid drumstick leaves (*Moringa oleifera*). J Food Res 2013;2:101–108
55 Friedman M. Nutritional value of proteins from different food sources. A review. J Agric Food Chem 1996;44:6–29
56 Sridhar R, Lakshminarayana G. Lipid classes, fatty acids, and tocopherols of leaves of six edible plant species. J Agric Food Chem 1993;41:61–63
57 Uddin MK, Juraimi AS, Hossain MS, Nahar MAU, Ali ME, Rahman MM. Purslane weed (Portulaca oleracea): a prospective plant source of nutrition, omega-3 fatty acid, and antioxidant attributes. ScientificWorldJournal 2014;2014:951019
58 Nutritional aspects of cardiovascular disease. Report of the Cardiovascular Review Group Committee on Medical Aspects of Food Policy. Rep Health Soc Subj (Lond) 1994;46:1–186

