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Nutritional composition, antioxidant activity, minerals and anti-nutritional factors of indigenous leafy vegetables of eastern India

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The underutilized leafy vegetables indigenous to eastern India constitute an integral part of food and nutrition of local population. In the current study, 22 indigenous species of leafy vegetables eaten by local people of eastern India were characterized by their nutritional and antioxidant profile. Most of these leafy vegetables showed great nutritional potential being rich in carotenoids (11.76±2.601 to 76.24±4.80 mg/100 g), ascorbic acid (3.01±0.241 to 156.92±4.37 mg/100 g), crude fiber (0.74±0.019 to 4.98±0.06%) and many essential minerals such as calcium (52.71±1.8 to 1114.2±9.1 mg/100 g), magnesium (28.87±4.70 to 566.0±4.1 mg/100 g), potassium (151.4±9.2 to 1465.38±4.0 mg/100 g), phosphorus (5.64±0.8 to 174.4±1.9 mg/100 g), iron (1.46±0.5 to 52.8±1.3 mg/100 g), zinc (0.17±0.2 to 5.91±0.4 mg/100 g), copper (0.11±0.06 to 1.4±0.1 mg/100 g) and manganese (0.35±0.2 to 80.7±2.0 mg/100 g). These indigenous leafy vegetables were also rich in antioxidants showing good DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity (17.43±1.673 to 1978.00±8.75 mg ascorbic acid equivalent antioxidant capacity (AEAC)/100 g), ferric reducing antioxidant potential (FRAP) (981.19±2.11to 5200.85±8.31 mg AEAC/100 g) and total phenol content (0.40±0.016 to 6.92±0.02 mg gallic acid equivalent (GAE)/100 g). Moreover, anti-nutritional compounds (oxalate, nitrate and tannins) in most of these indigenous leafy vegetables were found lower as compared to other commonly consumed leafy vegetables. Thus including these nutrients rich traditional leafy vegetables in our daily diet can be an excellent food-based approach for ensuring nutritional security.

Keywords: Eastern India, Leafy, Nutritional, Underutilized, Vegetables

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Since ancient times, people have used green leafy vegetables to ensure a secure and healthy life. Amaranth, spinach, fenugreek, coriander and other regularly consumed leafy vegetables have had their nutritional content listed in the National Institute of Nutrition's Food Composition tables[1]. Besides these there are also many other locally consumed indigenous leafy greens that can be found only in their specific season and little knowledge is accessible on the nutritional composition of these leafy vegetables. People who live in the regions where these underutilized leafy vegetables grow are the only ones who can eat them. Many of these lesser known native green leafy vegetables including *Asparagus officinalis, Citrullus lanatus, Basella alba, Ipomoea aquatica,* and *Moringa oleifera* are considered to be an excellent source of fibre, vitamins, and minerals including calcium, magnesium, iron, and potassium[2,3]. Additionally, these are excellent source of antioxidants and dietary fibre[4]. These leafy vegetables are also abundant in many nutraceutical compounds that are vital for the health of people and used primarily in accordance with traditional customs and knowledge.

Eastern Indian states like Jharkhand and Odisha, especially tribal region of the states are home to a large number of these traditional green leafy vegetables. As an essential component of the local population's diet and nutrition, these green vegetables play a significant role in the lives of the locals. Several of these local leafy vegetables have traditionally been utilized for their health-promoting, healing, and nutritional properties from ancient times. These are eaten as traditional specialties, and the sales of the additional produce enable a lot of rural families enhance their income.

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Since indigenous vegetables are usually rich in minerals, vitamins, dietary fibre and several bioactive compounds, these also assist in overcoming challenges of nutritional security[5]. Thus consuming these native leafy vegetables can be of tremendous help in fighting the malnutrition especially micronutrient deficiency predominant in the country and also to improve the socio-economic status of the society. More research on nutritional characterization and health benefits of these indigenous leafy greens is needed. Moreover, prior to popularizing any such species, it is necessary to identify and quantify any anti-nutritional factors present. Therefore, the goal of the current study was to examine the indigenous green leafy vegetables consumed in tribal belt of eastern India for their nutritional, antioxidant and anti-nutritional properties.

Materials and Methods

Study area

The current investigation was carried out in 2015-16, in the tribal region of states of Jharkhand and Odisha in eastern part of India. The area extends between 22°27'23 to 23°16'31N latitude and 85°23'50 to 85°47'5E longitude in Jharkhand and between 21.1102 -22.2604° N latitude, 84.4560 - 84.9167° E longitude in Odisha state and was selected based on larger percentage of native people living in the region whose daily diet constitute a lot of the lesser known leafy vegetables.

Methodology

Total 37 local markets in the area selected for the study were investigated at various times of the year because the kind and availability of leafy vegetables change significantly depending on the season. The primary objective of the survey was to determine potential underutilized leafy vegetable species consumed by local people of the region.

Preparation of samples for analysis

Samples of 22 species of indigenous green leafy vegetable were gathered from the marketplaces during surveys. 5 samples of every species were gathered from different marketplaces and combined for biochemical analysis. Analysis of moisture, vitamin C, total carotenoids, antioxidant activity and total phenols were carried out on fresh samples. For estimation of minerals, crude fibre and antinutritional components, dried powdered samples were used.

Moisture and crude fibre

The amount of moisture and crude fibre in the samples were analyzed following the standard protocol of AOAC (Association of Official Analytical Chemists)[6].

Ascorbic acid (vitamin C)

Ascorbic acid content of fresh leafy samples was also analyzed using standard AOAC method where titration with 6-dichlorophenol-indophenol dye was used to measure the amount of ascorbic acid[6].

Total carotenoids

A fresh leaf sample weighing 500 mg was thoroughly homogenized into 50 mL of pure ethanol followed by filtration using Whatman No. 42 filter paper. The filtrate was then diluted and the absorption was determined at wavelengths of 470, 649, and 665 nm. The calculations for total carotenoids content were then made on the basis of fresh weight[7].

Antioxidant activity

Two assays; 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and ferric reducing antioxidant potential (FRAP) were used to measure the antioxidant activity of the samples. Both of the tests are quick, easy to use, precise, and very consistent[8,9]. Both the assays used 80% methanol for extraction of samples.

DPPH assay

DPPH radical scavenging ability was used to assess the antioxidant capacity of fresh samples[10]. The DPPH assay depends on antioxidants reducing DPPH radicals in the presence of methanol, which results into reduced OD at 517 nm. The antioxidant capacity was calculated using the reduction in absorbance.

FRAP assay

The principle of FRAP assay is the reduction of ferric complex (no color) to ferrous complex (blue color) in acidic environment by the effect of electrons donated by antioxidants present in the extract[11]. The shift in absorption at 593 nm was used to calculate antioxidant activity.

Total phenols

Total phenols were quantified by Folin-Ciocalteu (FC) reagent method[12].

Minerals

Digestion for mineral analysis

Oven dried ground sample (1 g) was digested with 10 mL of diacid mixture (HNO_3: $HClO_4$, 9:4) at 80-100°C until clear. After cooling, the volume was adjusted to 100 mL with distilled water. The solution was filtered through Whatman No. 1 filter paper for analysis of minerals like phosphorus (P), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn).

Analysis of minerals

Total P content was measured using vanado-molybdophosphoric acid colorimetric method[13], K and Na were analysed using flame photometer[13]. Ca and Mg were measured using versanate method[14]. Fe, Mn, Cu and Zn were determined using atomic absorption spectrophotometer (Analyst-100, Perkin-Elmer, USA).

Anti-nutritional factor

Total oxalate content of the samples was analyzed using standard AOAC protocol[6]. Powdered sample (0.1 g) was added with 1 M HCl (30 mL) and incubated for 30 min. Then 5% $CaCl_2$ (0.5 mL) was mixed and stirred followed by centrifugation at 800 g for 15 min. Supernatant was removed and the pallet after washing twice with 0.35 M NH_4OH was dispersed in 0.5 M H_2SO_4. The temperature of the solution was kept at 60°C while titration was performed against 0.1 M $KMnO_4$ standard solution. Total oxalate was expressed in mg/100 g fresh weight (FW).

Nitrate content was analyzed using the method explained by IITA[15] in which 100 mg powdered sample was added with DW (10 mL) followed by incubation at 45°C for 1 h, cooling and centrifugation at 5000 rpm for 15 min. Then 0.2 mL of supernatant was mixed with 5% (w/v) salicylic acid sulphuric acid reagent (0.8 mL) and stirred properly. After 20 min, 2M NaOH was added to increase the pH over 12. The absorbance was recorded at 410 nm. The amount of nitrate (mg of NO_3/100 g) was estimated employing standard curve prepared using various conc. of KNO_3.

Tannin content was determined by Folin-Denis (FD) reagent method[6]. Powdered sample (0.5 g)

Was boiled for 30 min with 75 mL DW followed by centrifugation. The aliquote (1 mL) from the diluted supernatant was mixed with 75 mL DW, 5 mL FD reagent and 10 mL sodium-carbonate followed by making up the volume to 100 mL using DW. Tannin content was measured by observing the absorbance at 700 nm.

Statistical analysis

The data acquired in the current investigation were processed using completely randomized design (CRD) of one factor statistical analysis of variance (ANOVA). The critical difference value obtained at 5% levels of significance was employed to compare different formulations. Statistical ranks based on significant differences established by Duncan's multiple range tests are superscripted over the corresponding data values. Standard deviations were also calculated for all the biochemical parameters.

Results and Discussion

The study provided evidence of vast diversity of underutilized indigenous leafy vegetables in the tribal region of eastern India. These indigenous leafy vegetables are sold in two forms; fresh and dried. During the present investigation, 22 species of lesser known leafy vegetables consumed by indigenous people of these two states of eastern India were identified during market survey (Fig. 1).

Nutritional value

All the 22 species of indigenous leafy vegetables of eastern India identified during market survey were analyzed for their nutritional value which is depicted in Table 1. Moisture content among these leafy vegetables varied significantly being lowest in *Centella asiatica* (74.83±0.38%) and highest in *Portulaca oleracea* L. (93.54±0.137%). In a similar study comparable moisture content (71.74 to 98.20%) has also been reported in 8 local species of leafy greens of Assam state of India[16]. Ascorbic acid content in green leafy vegetables analyzed during present study varied significantly from 3.01±0.241 to 156.92±4.37 mg/100 g. Leaves of *Moringa oleifera* Lam. contained significantly higher amount of ascorbic acid (156.92±4.37 mg/100 g) as compared to all other species studied. Leafy vegetables such as *Cissus adnata* (118.07±2.91 mg/100 g), *Amaranthus dubius* (85.47±0.33 mg/100 g) and *Basella alba* (71.58±0.23 mg/100 g) were also found significantly richer in vitamin C as compared to other species. The findings are consistent with Sultanbawa & Sivakumar (2022)[17] who reported that fresh leaves of *Moringa* contain approximately 4 times higher vitamin C (220 mg/100 g) as compared to an orange (59.1 mg/100 g). Ascorbic acid (vitamin C) is a vital vitamin that is

Fig. 1 —Indigenous leafy vegetables of eastern India*; a) *Alternanthera sessilis* (Madranga), b) *Leucas aspera* (Guma/Gainsa), c) *Corchorus capsularis* (Jute saag), d) *Centella asiatica* (Beng), e) *Basella alba* (Poi), f) *Gmelina arborea* (Ghambari), g) *Amaranthus viridis* (Khada Saag), h) *Amaranthis dubius* (Lal Khada saag), i) *Amaranthus retroflexus* (Seriali), j) *Ipomoea aquatic* L. (Kalmi), k) *Cissus adnate* Roxb. (Khatta sag), l) *Colocasia esculenta* L. (Pechki), m) *Hygrophylla spinose* T. Anders (Muchari), n) *Moringa oleifera* Lam. (Munga), o) *Cucurbita moschata* L.(Kohra), p) *Oxalis corniculata* L. (Teenpatiya), q) *Polygonum plebeium* R. Br. (Chimti), r) *Portulaca oleracea* L. (Golgola), s) *Commelina benghalensis* L. (Kenna), t) *Hibiscus cannabinus* L. (Kudrum), u) *Polygonum glabrum* Willd (Nadi), v) *Trianthema monogyna* L. (Khapra)

*local names in parenthesis

essential for keeping a good healthy and improving immunity[18]. Comparatively lower vitamin C content (50 to 72 mg/100 g) was reported in a similar study on 13 traditional uncultivated green leafy vegetables of south-west Nigeria[19].

Carotenoids, known to be vitamin A precursors, are present in abundance in green leafy vegetables and help in reducing risk of cancers, cardiovascular diseases, cataract, and diseases related to low immune function[20]. Total carotenoids of the underutilized leafy vegetables of eastern India found under present study varied significantly from 11.76±2.601 mg/100 g in *Polygonum plebeium R. Br.* To 76.24±4.80 mg/100 g in *Alternanthera sessilis* which was comparable to those reported in seventy edible wild green leafy vegetables of South India (5.12 to 36.13 mg/100 g)[21]. Moreover, species such as *Amaranthus viridis* (62.24±2.66 mg/100 g), *Ipomoea aquatica* (46.13±6.82 mg/100 g), *Trianthema monogyna* L. (39.11±2.49 mg/100 g) and *Polygonum glabrum* Willd (36.99±2.54 mg/100 g) also contained significantly higher carotenoids content as compared to other leafy vegetables. Other researchers have also observed even higher variations in the total carotenoid content (4±1 to 494±22 µg/g FW) of 26 common green leafy vegetables of South-east Asia[22]. The crude fiber content of indigenous leafy vegetables under study varied significantly from 0.74±0.019% to 4.98±0.06%. Species such as *Amaranthus dubius* (4.98±0.06%), *Amaranthus viridis* (4.84±0.04%) and *Centella asiatica* (4.59±0.04%) were found significantly higher in fiber content. Consuming fibre reduces blood plasma cholesterol levels and improves bowel health[23].

Antioxidant activity and total phenols

DPPH radical-scavenging activity of the underutilized leafy vegetables species varied significantly among the leafy vegetables ranging from 17.43±1.673 mg AEAC/100 g in *Portulaca oleracea* L. to 1978.00±8.75 mg AEAC/100 g in *Hygrophylla spinosa*. Ferric-reducing antioxidant power (FRAP) of the leafy vegetables also varied significantly from 981.19±2.11 mg AEAC/100 g in *Portulaca oleracea* L. to 5200.85±8.31 mg AEAC/100 g in *Hygrophylla spinosa.* Statistical analysis showed that species such as *Hygrophylla spinosa* T. Anders, *Hibiscus cannabinus* L. and *Moringa oleifera* Lam. showed significantly higher antioxidant potential in both the assays (Table 1) and thus are definitely a good source of health promoting antioxidants. In a similar study, the DPPH antioxidant activity of the selected edible native plant species of Bangladesh ranged from 75.34% to 89.28%, whereas FRAP values ranged

Table 1 — Nutritional characterization of indigenous leafy vegetables of eastern India*

S. No.	Name of Leafy Vegetables	Moisture (%)	Ascorbic Acid (mg/100 g)	Carotenoids (mg/100 g)	Fiber (%)	Antioxidant Activity (mg AEAC/100 g)		Phenols (mg GAE/100 g FW)
						DPPH	FRAP	
1.	*Alternanthera sessilis*	81.37±0.38^{j}	42.73±0.48^{g}	76.24±4.80^{a}	1.19±0.04^{k}	998.84±7.27^{d}	2957.58±3.19^{h}	2.57±0.06^{g}
2.	*Leucas aspera*	80.01±0.19^{k}	20.30±0.38^{j}	31.20±2.00^{e}	1.19±0.04^{k}	892.49±3.35^{e}	3483.48±5.39^{e}	3.46±0.01^{e}
3.	*Amaranthus viridis*	87.69±0.16^{d}	21.37±0.52^{ij}	62.24±2.66^{b}	4.84±0.04^{b}	494.48±1.83^{h}	3567.29±8.28^{d}	1.92±0.02^{j}
4.	*Amaranthus dubius*	78.19±0.39^{l}	85.47±0.33^{c}	13.34±2.87^{m}	4.98±0.06^{a}	1290.52±4.29^{c}	2746.48±2.32^{j}	6.92±0.02^{a}
5.	*Basella alba*	86.82±0.35^{e}	71.58±0.23^{d}	20.39±3.06^{ijk}	1.75±0.01^{h}	388.12±5.37^{i}	1932.12±4.26^{q}	2.18±0.01^{i}
6.	*Amaranthus retroflexus*	85.97±0.46^{g}	23.50±0.49^{i}	12.22±2.47^{m}	2.21±0.02^{f}	568.60±4.38^{g}	2203.84±6.45^{k}	2.34±0.05^{h}
7.	*Centella asiatica*	74.83±0.38^{m}	28.85±1.20^{h}	20.80±1.85^{hij}	4.59±0.04^{c}	468.69±6.47^{h}	3400.93±7.41^{f}	2.87±0.03^{f}
8.	*Gmelina arborea*	82.08±0.18^{i}	45.94±0.24^{f}	20.61±2.57^{ijk}	1.87±0.03^{g}	399.40±6.12^{i}	2833.47±4.19^{i}	1.61±0.01^{k}
9.	*Corchorus capsularis*	80.01±0.19^{k}	55.55±0.38^{e}	27.82±3.12^{efg}	1.57±0.02^{i}	307.55±3.29^{j}	1938.66±5.98^{op}	1.85±0.01^{j}
10.	*Ipomoea aquatica*	90.07±0.32^{b}	11.00±0.18^{l}	46.13±6.82^{c}	0.99±0.03^{l}	260.00±9.04^{kl}	2078.03±6.94^{m}	2.66±0.01^{g}
11.	*Cissusadnata*	89.11±0.29^{c}	118.07±2.91^{b}	30.60±2.20^{ef}	2.32±0.13^{e}	465.00±7.44^{h}	2093.83±4.95^{l}	0.90±0.01^{n}
12.	*Colocasia esculenta*	88.69±0.26^{c}	9.56±1.25^{l}	15.60±3.12^{klm}	0.98±0.02^{l}	260.00±5.95^{kl}	4058.33±4.43^{c}	3.51±0.06^{e}
13.	*Hygrophylla spinosa*	88.00±0.35^{d}	4.82±0.33^{mnop}	25.81±3.99^{fgh}	1.18±0.01^{k}	1978.00±8.75^{a}	5200.85±8.31^{a}	6.64±0.21^{b}
14.	*Moringa oleifera*	79.72±0.30^{k}	156.92±4.37^{a}	31.00±1.91^{e}	1.40±0.05^{j}	1782.48±8.34^{b}	3558.44±7.39^{d}	5.94±0.25^{d}
15.	*Cucurbita moschata*	87.94±0.27^{d}	17.50±1.69^{k}	13.80±1.27^{lm}	1.52±0.04^{i}	236.00±5.93^{lm}	1033.56±4.92^{t}	1.09±0.05^{m}
16.	*Oxalis corniculata* L.	86.21±0.12^{fg}	4.66±0.32^{mnop}	13.33±1.97^{m}	0.87±0.03^{m}	270.00±7.20^{k}	1437.38±5.34^{s}	1.36±0.05^{l}
17.	*Polygonum plebeium* R. Br.	82.45±0.14^{i}	5.67±0.43^{mn}	11.76±2.60^{m}	2.75±0.05^{d}	164.07±4.00^{n}	2029.92±6.32^{n}	2.17±0.08^{i}
18.	*Portulaca oleracea* L.	93.54±0.14^{a}	3.01±0.24^{op}	25.07±2.95^{ghi}	0.74±0.02^{n}	17.43±1.67^{op}	981.19±2.11^{u}	0.40±0.02^{op}
19.	*Commelina benghalensis* L.	87.59±0.33^{d}	5.89±0.53^{m}	30.91±4.68^{ef}	1.76±0.03^{h}	740.31±30.55^{f}	3162.02±8.65^{g}	1.44±0.08^{l}
20.	*Hibiscus cannabinus* L.	83.62±0.11^{h}	46.73±1.55^{f}	18.57±0.88^{jkl}	1.71±0.04^{h}	1780.60±76.33^{b}	4207.74±4.54^{b}	6.35±0.04^{c}
21.	*Polygonum glabrum* Willd.	86.69±0.32^{ef}	3.61±0.19^{nop}	36.99±2.54^{d}	1.38±0.03^{j}	215.13±6.99^{m}	2746.03±3.10^{j}	1.79±0.02^{j}
22.	*Trianthema monogyna* L.	90.25±0.462^{b}	3.89±0.25^{mnop}	39.11±2.49^{d}	1.24±0.03^{k}	150.56±3.00^{n}	1537.83±6.18^{r}	1.12±0.03^{m}
	CD (0.05)	*0.488*	*2.160*	*5.137*	*0.072*	*30.306*	*9.525*	*0.131*

* Five samples were pooled from different markets for each leafy vegetable and the analysis was done in triplicate

between 262.91±2.35 and 845.87±5.37 µmol $Fe^{(2+)}$/g[24]. These traditional green leafy vegetables may have strong anti-inflammatory and ethno-medicinal properties due to their strong anti-oxidant properties which could explain their widespread use in the diet of the local people[25].

A significant variation in the total phenol content of leafy vegetables was also observed among the different species ranging from 0.40±0.02 mg GAE/100 g in *Portulaca oleracea* L. to 6.92±0.02 mg GAE/100 g in *Amaranthus dubius*. The values were comparatively lower to those obtained in selected culinary microgreens (14.6 to 73.6 mg GAE/100 g) in another study[26]. The primary antioxidant components of leafy greens are phenolic compounds since several studies have already established a linear association between antioxidant capacity and the total amount of phenols[25,27].

Minerals

Mineral contents of the indigenous leafy vegetables under study have been presented in Table 2. A significant variation in Ca content was observed among different species and it varied from 52.71±1.8 to1114.2±9.1 mg/100 g, highest being in *Moringa oleifera* (1114.2±9.1 mg/100 g) followed by *Polygonum glabrum* Willd. (1073.39±5.87 mg/100 g), *Commelina benghalensis* L. (1033.7± 4.22 mg/100 g) and *Hygrophylla spinosa* (978.5±9.3 mg/100 g). Mg content varied significantly among the species and

Table 2 — Minerals content of indigenous leafy vegetables of eastern India*

SN	Name of Leafy Vegetables	Minerals (mg/100 g)								
		Ca	Mg	P	K	Na	Fe	Zn	Cu	Mn
1.	*Alternanthera sessilis*	260.83 ± 6.4^{l}	201.21 ± 3.9^{j}	13.07 ± 1.5^{lm}	877.49 ± 6.6^{h}	11.63±1.9jk	10.71 ± 1.5^{cd}	1.43 ± 0.2^{fgh}	0.29 ± 0.09^{ef}	9.94 ± 0.5^{b}
2.	*Leucas aspera*	279.85 ± 3.1^{k}	71.96 ± 2.9^{q}	17.16 ± 1.4^{kl}	1077.41 ± 4.2^{d}	1.14 ± 0.8^{n}	1.46 ± 0.5^{h}	1.06 ± 0.2^{hi}	0.25 ± 0.17^{efgh}	2.25 ± 0.4^{gh}
3.	*Amaranthus viridis*	369.32 ± 4.0^{h}	118.18 ± 1.5^{l}	11.09 ± 2.1^{m}	883.91 ± 3.9^{h}	12.54 ± 1.9^{ijk}	4.93 ± 1.2^{ef}	0.83 ± 0.3^{ij}	0.24 ± 0.06^{efgh}	1.33 ± 0.2^{ijk}
4.	*Amaranthus dubius*	654.41 ± 2.6^{e}	287.94 ± 4.3^{f}	18.86 ± 1.3^{k}	$1153.95c\pm2.4^{c}$	25.79 ± 3.4^{e}	9.63 ± 1.6^{d}	1.84 ± 0.3^{def}	0.37 ± 0.08^{de}	2.38 ± 0.1^{g}
5.	*Basella alba*	52.71 ± 1.8^{s}	268.83 ± 5.2^{g}	13.22 ± 0.9^{lm}	1465.38 ± 4.0^{a}	42.35 ± 1.2^{cd}	13.34 ± 2.8^{c}	0.76 ± 0.2^{ij}	0.15 ± 0.09^{fgh}	1.53 ± 0.3^{hij}
6.	*Amaranthus retroflexus*	280.55 ± 5.6^{k}	218.83 ± 6.6^{i}	8.31 ± 1.8^{mn}	1216.19 ± 7.3^{b}	6.01 ± 1.8^{lm}	9.31 ± 1.4^{d}	0.82 ± 0.2^{ij}	0.24 ± 0.08^{efgh}	0.68 ± 0.2^{kl}
7.	*Centella asiatica*	352.33 ± 5.0^{i}	301.99 ± 1.2^{e}	5.64 ± 0.8^{n}	926.12 ± 4.9^{f}	165.56 ± 3.3^{a}	28.4 ± 2.6^{b}	5.91 ± 0.4^{a}	0.28 ± 0.07^{ef}	9.93 ± 0.3^{b}
8.	*Gmelina arborea*	294.5 ± 2.8^{j}	256.51 ± 1.8^{h}	10.46 ± 2.1^{mn}	849.32 ± 9.0^{i}	17.85 ± 1.4^{fgh}	4.54 ± 1.1^{efg}	1.16 ± 0.2^{ghi}	0.17 ± 0.05^{fgh}	2.46 ± 0.2^{g}
9.	*Corchorus capsularis*	167.48 ± 1.7^{op}	83.74 ± 3.5^{mn}	9.87 ± 1.7^{mn}	722.24 ± 4.3^{k}	4.53 ± 2.5^{mn}	2.98 ± 0.8^{fgh}	1.13 ± 0.2^{ghi}	0.18 ± 0.07^{fgh}	0.61 ± 0.1^{kl}
10.	*Ipomoea aquatica*	396.5 ± 5.1^{g}	444.2 ± 4.8^{b}	174.4 ± 1.9^{a}	545.2 ± 5.6^{n}	47.33 ± 3.9^{b}	52.8 ± 1.3^{a}	4.4 ± 0.4^{c}	1.1 ± 0.10^{c}	5.3 ± 0.3^{d}
11.	*Cissus adnata*	234.51 ± 2.2^{n}	87.34 ± 2.3^{m}	8.96 ± 1.1^{mn}	663.65 ± 2.4^{l}	15.99 ± 3.2^{ghi}j	9.14 ± 1.1^{d}	1.83 ± 0.4^{def}	0.27 ± 0.04^{efg}	2.32 ± 0.1^{gh}
12.	*Colocasia esculenta*	583.5 ± 4.2^{f}	317.3 ± 2.3^{d}	91.8 ± 1.6^{d}	1021.2 ± 10.2^{e}	20.13 ± 2.3^{fg}	11.0 ± 0.7^{cd}	5.2 ± 0.4^{b}	1.4 ± 0.1^{b}	80.7 ± 2.0^{a}
13.	*Hygrophylla spinosa*	978.5 ± 9.3^{d}	347.8 ± 3.2^{c}	42.4 ± 2.4^{g}	895.2 ± 6.6^{g}	46.23 ± 3.1^{bc}	9.2 ± 0.6^{d}	5.6 ± 0.2^{ab}	2.2 ± 0.09^{a}	5.0 ± 0.3^{de}
14.	*Moringa oleifera*	1114.2 ± 9.1^{a}	566.0 ± 4.1^{a}	55.2±3.2f	585.0 ± 3.3^{m}	19.07 ± 1.8^{fgh}	9.6 ± 0.1^{d}	1.5 ± 0.2^{efg}	0.5 ± 0.06^{d}	2.8 ± 0.2^{g}
15.	*Cucurbita moschata*	283.2 ± 1.6^{k}	320.9 ± 1.0^{d}	81.8 ± 2.0^{e}	672.5 ± 3.3^{l}	16.15 ± 1.4^{ghij}	6.2 ± 0.2^{e}	4.2 ± 0.3^{c}	1.0 ± 0.17^{c}	4.1 ± 0.2^{f}
16.	*Oxalis corniculata* L.	126.2 ± 8.2^{q}	85.6 ± 8.6^{mn}	36.97 ± 3.4^{h}	259.2 ± 6.7^{s}	24.9 ± 1.2^{e}	9.61 ± 0.8^{d}	0.76 ± 0.12^{ij}	0.45 ± 0.1^{d}	0.65 ± 0.1^{kl}
17.	*Polygonum plebeium* R. Br.	247.3 ± 5.3^{m}	118.3 ± 1.5^{l}	52.1 ± 2.7^{f}	151.4 ± 9.2^{t}	9.1 ± 1.1^{kl}	11.66 ± 0.5^{cd}	0.58 ± 0.1^{jk}	0.20 ± 0.15^{fgh}	0.84 ± 0.1^{jkl}
18.	*Portulaca oleracea* L.	68.39 ± 4.5^{r}	75.87 ± 3.6^{opq}	45.98 ± 4.3^{g}	512 ± 6.4^{op}	38.39 ± 2.8^{d}	1.99 ± 0.8^{gh}	0.17 ± 0.2^{k}	0.12 ± 0.05^{gh}	0.35 ± 0.2^{l}
19.	*Commelina benghalensis* L.	1033.7 ± 4.22^{c}	221.2 ± 5.77^{i}	165 ± 8.4^{b}	410 ± 9.07^{q}	21.92 ± 6.78^{ef}	11.25 ± 4.64^{cd}	2.17 ± 0.3^{d}	0.26 ± 0.04^{efgh}	4.4 ± 0.3^{ef}
20.	*Hibiscus cannabinus* L.	231.37 ± 8.43^{n}	28.87 ± 4.70^{r}	31.93 ± 3.28^{i}	158.23 ± 8.4^{t}	15.23 ± 3.12^{hij}	3.78 ± 1.7^{efgh}	1.87 ± 0.2^{de}	0.14 ± 0.14^{fgh}	1.98 ± 0.3^{ghi}
21.	*Polygonum glabrum* Willd.	1073.39 ± 5.87^{b}	79.33 ± 6.98^{nop}	112.65 ± 4.19^{c}	745.84 ± 3.6^{j}	17.89 ± 1.2^{fgh}	2.87 ± 1.7^{fgh}	0.41 ± 0.1^{jk}	0.17 ± 0.09^{fgh}	8.43 ± 0.5^{c}
22.	*Trianthema monogyna* L.	57.54 ± 4.47^{s}	153.98 ± 4.18^{k}	24.64 ± 3.56^{j}	318.35 ± 10.32^{r}	16.86 ± 3.2^{ghi}	$4.12\pm2.37_{efgh}$	0.44 ± 0.2^{jk}	0.11 ± 0.06^{h}	0.46 ± 0.2^{l}
	CD (0.05)	*8.754*	*7.053*	*4.943*	*10.664*	*4.528*	*2.788*	*0.422*	*0.158*	*0.825*

* Five samples were pooled from different markets for each leafy vegetable and the analysis was done in triplicate

ranged from 28.87±4.70 to 566.0±4.1 mg/100 g. Species like *Moringa oleifera* (566.0±4.1 mg/100 g), *Ipomoea aquatica* (444.2±4.8 mg/100 g), *Hygrophylla spinosa* (347.8±3.2 mg/100 g) were found to contain significantly higher amount of Mg. Similar values of Ca (2.6 to 982.9 mg/100 g) in twelve indigenous green leafy vegetables of Thailand[28] and Mg (30.30 to 201.20 mg/100 g) in 21 native species of leafy greens found in North Eastern India have been reported previously[29].

The Fe content of leafy vegetables analyzed during current investigation ranged between 1.46±0.5 mg/100 g in *Leucas aspera* and 52.8±1.3 mg/100 g in *Ipomoea aquatica*. *Ipomoea aquatica* (52.8±1.3 mg/100 g), *Centella asiatica* (28.4±2.6 mg/100 g) and *Basella alba* (13.34±2.8 mg/100 g) were found significantly richer in iron content as compared to other species. Comparable Fe values have also been reported in lesser known leafy vegetables of West Bengal, India (5.4-1.5 mg/100 g)[30] and in some common and wild leafy vegetables of Nigeria (21.30 - 33.40 mg/100 g)[31]. The average recommended dietary allowances (RDA) of these minerals; Ca (1000 mg per day), Mg (400 mg per day) and Fe (8 mg per day) show that the indigenous leafy vegetables under present investigation can improve diet significantly in terms of these mineral as some of these can provide even upto 100% of RDA of these minerals.

The K content of these indigenous leafy vegetables varied significantly and ranged from 151.4±9.2 mg/100 g to 1465.38±4.0 mg/100 g. *Basella alba* contained significantly higher amount of K (1465.38±4.0 mg/100 g) followed by *Amaranthus retroflexus* (1216.19±7.3 mg/100 g) and *Amaranthus dubius* (1153.95±2.4 mg/100 g). The results are in conformity with previously reported high levels of several minerals in *Basella alba*, a widespread but underutilized leafy vegetable of Asian region[32].K content in similar range (221.7 to 1291.3 mg/100 g) has also been reported in twelve indigenous wild leafy vegetables of Thailand[28]. Na) content varied significantly from 1.14±0.8 mg/100 g to 165.56±3.3 mg/100 g and was fairly low except for *Centella asiatica* which contained a significantly higher amount of Na (165.56±3.3 mg/100 g) than the other species. Comparable Na levels (5.0–111.1 mg/100 g) were reported in a similar study on lesser known leafy vegetables of India[30]. The ratio of K and Na in a food is closely correlated with high blood pressure and coronary artery disease Na increases blood pressure while K decreases it[33]. Leafy vegetables species namely *Ipomoea aquatica, Commelina benghalensis* L. and *Polygonum glabrum Willd.* contained significantly higher amount of Pand *Centella asiatica*, *Hygrophylla spinosa* and *Colocasia esculenta* had significantly higher levels of Zn content. Cu content under the study also varied significantly and was found significantly higher in species such as *Hygrophylla spinosa, Colocasia esculenta, Ipomoea aquatic* and *Cucurbita moschata*. Manganese (Mn) content was substantially higher in *Colocasia esculenta* (80.7±2.0 mg/100 g), for rest of the species it ranged from 0.35±0.2 to 9.94±0.5 mg/100 g.

Anti-nutritional factors

Anti-nutritional factors such as oxalate, nitrate and tannin levels present in the indigenous leafy vegetable species under study have been given in Table 3. Oxalic acid inhibits the uptake of calcium through formation of calcium oxalate and also aid the formation of kidney stones[34,35]. The oxalate content in the leafy vegetables showed a significant variation and ranged from 99.25±5.318 mg/100 g FW in *Polygonum plebeium* R. Br. to 682.13±27.97 mg/100 g FW in *Centella asiatica.* The oxalate content in all the leafy vegetables analyzed under present study except two; *Centella asiatica and Hygrophylla spinosa* were found significantly lower than those reported in popular leafy greens such as spinach (658 mg/100 g)[1].

Amount of nitrate present in a leafy vegetable is not toxic but its metabolic products like nitrite, nitric-oxide and N-nitroso compounds are hazardous to health and raise a health concern[36]. The nitrate content in the leafy vegetables under present study also varied significantly between142.16±1.61 mg/100 g FW in *Alternanthera sessilis* and 491.95±6.65 mg/100 g FW in *Centella asiatica* (broad leaved). Other than *Centella asiatica*, species with significantly greater nitrate contents included *Polygonum glabrum* Willd. and *Colocasia esculenta*.

Tannins adversely affect protein digestibility. The tannin content of the underutilized leafy vegetables under present study also showed a significant variation and fluctuated between 2.14±0.198 mg/100 g FWand 20.20±0.659 mg/100 g FW. Species such as *Centella asiatica* and *Moringa oleifera* although contained significantly higher levels of tannin among the species studied, the amount was considerably lower as compared to those found in some commonly eaten leafy vegetables such as spinach, cauliflower,

Table 3 — Antinutritional factors in indigenous leafy vegetables of eastern India*

S.No.	Name of Leafy Vegetables	Total oxalate (mg/100 g FW)	Nitrate (mg/100 g FW)	Tannin (mg /100 g FW)
1.	*Alternanthera sessilis*	145.50 ± 7.14^{jk}	142.16 ± 1.61^{q}	15.16 ± 0.23^{b}
2.	*Leucas aspera*	130.32 ± 8.28^{kl}	154.22 ± 2.77^{n}	14.26 ± 0.34^{c}
3.	*Amaranthus viridis*	151.56 ± 6.20^{jk}	174.43 ± 2.09^{l}	3.58 ± 0.87^{m}
4.	*Amaranthus dubius*	198.39 ± 4.26^{hi}	183.35 ± 3.13^{j}	8.61 ± 0.35^{e}
5.	*Basella alba* (small leaved)	589.77 ± 12.21^{b}	340.59 ± 5.41^{e}	6.84 ± 0.18^{fg}
6.	*Amaranthus retroflexus*	209.40 ± 22.18^{hi}	167.98 ± 3.05^{m}	5.72 ± 0.01^{ij}
7.	*Centella asiatica* (broad leaved)	682.13 ± 27.97^{a}	491.95 ± 6.65^{a}	20.20 ± 0.66^{a}
8.	*Gmelina arborea*	154.70±19.23jk	213.87 ± 3.04^{h}	8.62 ± 0.69^{de}
9.	*Corchorus capsularis*	528.14 ± 14.83^{c}	186.30 ± 2.70^{j}	6.64 ± 0.25^{fgh}
10.	*Ipomoea aquatica*	430.73 ± 27.97^{e}	181.07 ± 2.77^{jk}	4.80 ± 0.14^{kl}
11.	*Cissus adnata*	179.33 ± 13.98^{ij}	145.32 ± 1.99^{op}	4.27 ± 0.03^{lm}
12.	*Colocasia esculenta*	458.67 ± 27.97^{de}	401.14 ± 7.49^{c}	9.42 ± 0.60^{d}
13.	*Hygrophylla spinosa*	668.17 ± 36.99^{a}	352.90 ± 4.96^{d}	8.45 ± 1.00^{e}
14.	*Moringa oleifera* (Leaves)	263.13 ± 13.98^{g}	231.19 ± 1.68^{g}	19.58 ± 0.42^{a}
15.	*Cucurbita moschata*	291.07 ± 27.97^{fg}	243.35 ± 4.07^{f}	5.40 ± 0.53^{jk}
16.	*Oxalis corniculata* L.	139.67 ± 6.13^{k}	151.90 ± 2.45^{n}	7.02 ± 0.35^{fg}
17.	*Polygonum plebeium* R. Br.	99.25 ± 5.32^{lm}	175.75 ± 2.13^{kl}	6.44 ± 0.53^{ghi}
18.	*Portulaca oleracea* L.	207.27 ± 27.97^{hi}	244.10 ± 2.86^{f}	2.14 ± 0.20^{n}
19.	*Commelina benghalensis* L.	87.15 ± 19.58^{m}	157.62 ± 2.29^{n}	7.41 ± 0.25^{f}
20.	*Hibiscus cannabinus* L.	301.07 ± 30.15^{f}	204.76 ± 3.74^{i}	15.88 ± 0.82^{b}
21.	*Polygonum glabrum* Willd.	472.63 ± 27.97^{d}	420.46 ± 6.49^{b}	5.86 ± 0.22^{hij}
22.	*Trianthema monogyna* L.	222.58 ± 11.67^{h}	165.49 ± 2.99^{m}	3.63 ± 0.39^{m}
	CD (0.05)	*33.924*	*6.319*	*0.804*

* Five samples were pooled from different markets for each leafy vegetable and the analysis was done in triplicate

amaranth, lettuce and radish leaves (37.79±0.03 to 43.7±0.02 mg/100 g)[37]. However, in another study much higher tannin levels (138.27-892.19 mg/100 g) were discovered in several of the identified underutilised vegetables of Ethiopia[38]. Thus although majority of the leafy vegetables under study contain antinutritional factors within the safer limits, presence of some of these antinutrient factors indicates that eating excessive amount of some of these leafy vegetables can negatively affect the health, however these anti nutritional factors might be readily removed or decreased by soaking, boiling or frying of these vegetables before consumption[39].

Conclusion

Thus, it can be concluded that the 22 species of indigenous leafy vegetables identified during market survey of tribal parts of eastern India showed great nutritional potential being rich in carotenoids, vitamin C, fiber and many vital minerals such as Ca, Mg, K, Fe etc. Majority of these leafy vegetables contain lower amount of antinutrient components as compared to commonly consumed leafy vegetables. Moreover, these lesser known leafy vegetables also have great antioxidant potential and hence support good health. One food-based strategy for guaranteeing the intake of these nutrients is to boost the consumption of these leafy vegetables, which are widely recognized to be excellent sources of mineral, vitamins and dietary fibre. Prior to the disappearance of these treasured species from the rich biodiversity of our nation, it is imperative that considerable efforts be made to spread the health advantages of these lesser known indigenous leafy vegetables to the people across the country.

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Conflict of Interest

There is no conflict of interests.

Author Contributions

AS, RSP and BPB conceptualized and designed the experiments.AS, RSP, SKN and AKS performed the experiments. AS and RSP analyzed the data. AS, AKS and BPB provided materials, chemicals and analysis tools. AS, RSP and AKS wrote the paper.

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