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## RESEARCH ARTICLE

### NUTRITIONAL PROFILING OF SELECTED UNDERUTILIZED LEAFY VEGETABLES FOR FOOD VALUE

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# INTRODUCTION

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India boasts a number of species of edible leafy vegetables, some of which have no English names and are only recognized by their native names. India is blessed with a range of natural environments, diverse climates and varying seasons. These leafy veggies are consumable and are also known as unconventional greens or underutilized leafy vegetables. Everyday green vegetable preparations are consumed in Indian households. These neglected greens are affordable, simple to prepare and composed of considerable amount of vitamins, minerals, protein and other nutrients.

The presence of poisonous and anti-nutritional principles is the fundamental issue with nutritional exploitation of underused greens. Phytates, nitrate, oxalate and saponin are frequently found in high concentrations in leafy greens (Gupta et al., 1989). Leafy vegetables are a powerful source of phytochemicals that promote health. People can use them to treat and prevent a various deficiency conditions. Underutilized leafy vegetables have been focused with research attention for their nutrition quality and also to their neutraceutical value. To maintain a balance between the world's population expansion and agricultural output, researchers have recently looked for lesser-known leafy crops, many of which have the potential to be important as human food. The abundance of low-cost edible green leafy vegetables, as well as their favourable characteristics, spark interest in researching the nutritional value and anti-nutritional elements of a few particular non-conventional green leafy vegetables, such as Indian pennywort, sessile joyweed, Asiatic dayflower, Indian sorrel, chicory leaves etc and also their medicinal properties. Practically, there is no information available on the nutritive value of underutilized foods, which may contribute significantly to the nutrient intake of rural population. There are many vegetables available in this part of the country, whose nutritional profile is yet to be documented. More systematic study on these vegetables is required in order to fully utilize them. The nutritional dilemma in many countries, with many children and adults suffering from micronutrient deficiencies, is a strong motivation for determining the nutritional composition of traditional foods. In this background, the present study was planned to systematically analyse and document the nutrient content of Centella asiatica L., Alternanthera sessilis L., Amaranthus cruentus L., Celosia argentea L., Commelina communis L., Oxalis carniculata L., Hibiscus cannabinus L. This exploration can help to address malnourishment from eating a diet in which one or more nutrients are either not enough. It may involve calories, protein, carbohydrates, vitamins or minerals. Standard protocols for biochemical analysis are employed for nutrient analyses.

# MATERIALS AND METHODS

Preparation of dried leaf powders: Leafy vegetables were gathered, brought to the laboratory and thoroughly sorted. Removed unwanted parts and washed with tap water followed by distilled water rinsing. Then, leafy vegetables were dried in heat pump drier at  $45^{\circ}$ C temperature till the final moisture reaches constant weight in order retain maximum amount of nutrient components. For further study, the dried leafy vegetables were ground into a powder using a food processor, sieved using a 0.5 mm mesh size sieve, and then packaged in aluminium pouches

#### Analysis of nutritional quality of dried leaf powders

Phenols and flavonoids ( $\mu$ g 100 g<sup>-1</sup>): With a few minor adjustments, the individual phenolic acids and flavonoids for HPLC-MSMS analysis were isolated using 80% methanol, as previously reported by Weidner et al. (2000) and Chen et al. (2001). After homogenizing 10 g of the material in 80% methanol, the volume was increased to 50 mL by centrifuging the mixture. After being vacuum-sealed until almost dry at 45°C, 20 mL of the extract was diluted with 5 mL of water. Later extracted three times using petroleum ether, and then using a separating funnel, in 40 mL of ethyl acetate. The produced aqueous layer was disposed of, and the ethyl acetate extract was vacuum-dried to room temperature. 4 mL of 2N NaOH was added to the dry residue, and it was left to hydrolyze for the entire night. The extract was re-extracted using 50 mL of ethyl acetate after being acidified to pH 2 with 5 mL of 2N HCl. Using 25 milliliters of 0.1N NaHCO3, the ethyl acetate layer was extracted twice more. Before being injected into the HPLC-MSMS system for flavonoid estimation, the residue from the complete evaporation of the ethyl acetate layer containing the flavonoids was dissolved in

2 mL of MS grade methanol and filtered through a 0.2 μm nylon filter. After adding 5 mL of 2N HCl to the aqueous layer to further acidify it to pH 2, the layer was extracted three times using 25 mL of ethyl acetate. The ethyl acetate layer was then completely dried in a rotary evaporator, and the residue was dissolved in 2 mL of MS grade methanol before being injected into the HPLC-MSMS system to estimate phenolic acid.

Vitamins (ng  $100g^{-1}$ ): The method for extracting fat and water-soluble vitamins was adhered to, as detailed by Santos et al (2012).

Extraction of water soluble vitamins: 25 mL of 10 mM ammonium acetate: methanol 50:50 (v/v) containing 0.1% BHT was used to extract 5 g of the leaf powder. The samples were homogenized in a micro grinder for five minutes and vigorously shaken before being in a sonication bath set at  $25^\circ$ C for fifteen to twenty minutes. The sample was centrifuged for 15 minutes at 14000 rpm, and the supernatant was removed. The leftover material was saved for additional analysis, and the volume was adjusted after filtering the material through a 0.45 μm nylon filter. To find the water soluble vitamin content, 2 mL of the supernatant was dried into a nitrogen stream, reconstituted in 1 mL of mobile phase, filtered through a 0.2 μm nylon filter, and then injected into an HPLC-MS/MS.

# RESULTS

The study on individual phenolic acids profile revealed highest contents of Gentisic acid (241157.76 µg/100 g), Sinapic acid (43056.69  $\mu$ g/100 g), Ellagic acid (116.32  $\mu$ g/100 g) in T<sub>1</sub>, Benzoic acid (732.67 µg/100 g), Vanillic acid (2596.45  $\mu$ g/100 g) in T<sub>2</sub>, Ferulic acid (818205.36  $\mu$ g/100 g) in T<sub>3</sub>, Gallic acid (88019.44 µg/100 g), t-Cinnamic acid (2056.73  $\mu$ g/100 g) in T<sub>5</sub>, p-hydroxy benzoic acid (2285.22  $\mu$ g/100 g), 3-Hydroxy benzoic acid (2275.58 µg/100 g), Syringic acid (443.50  $\mu$ g/100 g), Chlorogenic acid (3327.92  $\mu$ g/100 g) in T<sub>6</sub> and Salicylic acid (1166075.62 µg/100 g), Protocatechuic acid (229322.24 µg/100 g), p-Coumaric acid (1722963.55 µg/100 g), o-Coumaric acid (881600.55 µg/100 g), Caffeic acid (451612.98  $\mu$ g/100 g) in T<sub>7</sub> respectively (Table 1 a, b, c). The study on individual flavonoids profile revealed highest contents of Catechin (2812.27  $\mu$ g/100 g) in T<sub>1</sub>, Apigenin (271.71  $\mu$ g/100 g), Fisetin (2.47  $\mu$ g/100 g) in T<sub>2</sub>, Naringenin (2668.13  $\mu$ g/100 g), Eriodictyol (3.98  $\mu$ g/100 g) in T<sub>3</sub>, Myricetin (78351.26 µg/100 g), Epicatechin (877.37 µg/100 g) in T4, Galangin (20.32 µg/100 g), Kaemperol (89.64 µg/100 g), Luteolin (5339.79  $\mu$ g/100 g) in T<sub>5</sub>, Hesperetin (806.25 µg/100 g), Quercetin (4144.99 µg/100 g), Rutin (17742.85  $\mu$ g/100 g) in T<sub>6</sub> respectively (Table 2 a, b c). Water soluble vitamin profiling in selected underutilized leafy vegetables revealed highest contents of Pantothenicacid (81916.80 ng/100 g), Thiamine (12111.64 ng/100 g) in  $T_2$ , Niacin (4182.11) ng/100 g), Pyridoxin (6118.35 ng/100 g), Riboflavin (3000.23 ng/100 g) in  $T_4$ , Folic acid (65.91 ng/100 g) in  $T_5$ , Biotin (34844.97 ng/100 g) and Cyanocobalamine (10044.48 ng/100 g) in  $T_7$  respectively (Table 3). Analogously, individual fatsoluble vitamin profiling in underutilized leafy vegetables revealed highest contents of Vitamin K2 (6304.08 ng/100 g) in T5, Vitamin D1 (666.03 ng/100 g), Vitamin D2 (7344.44 ng/100 g), Tocopherol (1809398.80 ng/100 g), Vitamin K1 (153131.07 ng/100 g) respectively (Table 4).



#### Table 1 a. Phenols profiling of selected underutilized leafy vegetables by HPLC

#### Table 1 b: Phenols profiling of selected underutilized leafy vegetables by HPLC



#### Table 1 c. Phenols profiling of selected underutilized leafy vegetables by HPLC



#### Table 2 a. Flavonoids profiling of selected underutilized leafy vegetables by HPLC



#### Table 2 b: Flavonoids profiling of selected underutilized leafy vegetables by HPLC







Table 3. Water soluble vitamins of selected underutilized leafy vegetables by HPLC

Treatments	Niacin	Pyridoxin	Pantothenicacid	<b>Biotin</b>	Thiamine	Riboflavin	Folic acid	Cyanocobalamine
	(ng/100 g)	(ng/100 g)	(ng/100 g)	(ng/100 g)	(ng/100 g)	(ng/100 g)	(ng/100 g)	(ng/100 g)
Centella asiatica L.	$2795.41^{\circ}$	$4062.39^{\circ}$	$40140.62^{\circ}$	$759.32^{\circ}$	6552.29 <sup>b</sup>	781.58 <sup>d</sup>	$32.38^{b}$	$4745.65$ <sup>d</sup>
Alternanthera sessilis L.	$2751.39^{\circ}$	5806.51 <sup>b</sup>	81916.80 <sup>a</sup>	$755.78^{\circ}$	$12111.64^a$	$1403.73^{\rm b}$	40.47 <sup>b</sup>	5266.80°
Amaranthus cruentus L.	2531.28 <sup>d</sup>	1955.11	81698.04 <sup>a</sup>	$721.14^{\circ}$	84.31	$1309.48^{\circ}$	$39.32^{b}$	$5421.11^{\circ}$
Celosia argentea L.	4182.11 <sup>a</sup>	$6118.35^{\circ}$	$40768.26^{b}$	$789.25^{\circ}$	$149.87$ <sup>d</sup>	3000.23 <sup>a</sup>	$23.12^{\circ}$	5234.78°
Commelina communis L.	$3521.78^b$	$2869.42^{\text{d}}$	$40144.71$ °	$1598.53^b$	$524.56^{\circ}$	$558.74^{\circ}$	$65.91^{\circ}$	5359.97°
Hibiscus cannabinus	$2201.11^d$	$1995.37^{\circ}$	32431.02 <sup>d</sup>	$1506.86^{\rm b}$	127.63	$831.46^{\rm d}$	$26.59^\circ$	$8681.93^{b}$
Oxalis carniculata L.	$2905.46^{\circ}$	$2316.95^{\circ}$	$10269.24^{\circ}$	34844.97 <sup>a</sup>	152.22	$133.35^t$	$50.88^{b}$	$10044.48^a$
Mean	2984.08	3589.18	46766.96	5853.96	2814.65	1145.51	278.68	6393.53
S. Em $(\pm)$	109.50	74.03	571.92	44.86	83.82	12.08	2.00	57.31
$CD$ at $1\%$	460.99	311.69	2407.75	188.87	352.88	50.88	8.34	241.29

Table 4. Fat soluble vitamins of selected underutilized leafy vegetables by HPLC



### DISCUSSION

Phenols ( $\mu$ g g-1 FW): Plant-based foods naturally contain polyphenols; these compounds have wide range of complex structures. The basic monomer in polyphenols is phenolic ring and generally these are classified as phenolic acids and phenolic alcohols. Depending upon the strength of phenolic ring, polyphenols can be classified in many classes, but the main classes in the polyphenols are phenolic acids, flavonoids, stiblins, phenolic alcohols and lignans. Bioactive compounds are the phytochemicals involved in protection of human health against the chronic degenerative ailments. Polyphenols are the group of biologically active compounds in plant-based foods. These compounds are embedded in human diet and originate from plants like fruits, vegetables, cereals and coffee. Polyphenols are also known as a preventive for the degenerative diseases. Investigations on polyphenols delayed due their particular characteristic, structural complexity. Most frequent antioxidants in our diet are polyphenols. These hinder the oxidative change in low density lipoprotein and this is the basic mechanism in endothelial lesions taking place in atherosclerosis.

Explorations witnessed the positive role of polyphenols in remedy of cardiovascular disease, osteoporosis, neurogenerative disease, cancer and diabetes mellitus. Most of the times, these phenolic compounds are analyzed by HPLC (Giusti et al., 1999; Vagiri et al., 2012), coupled with diode array detector and mass spectrometer (Revilla et al., 1999). In the present investigation, eighteen phenolic compounds were identified by comparing their retention times and mass spectra with respective standards. The perusal of mean values indicated that phenolic acids viz., Benzoic acid, p-hydroxy benzoic acid, Salicylic acid, 3-Hydroxy benzoic acid, t-Cinnamic acid, 2,4-dihydroxybenzoic acid, Gentisic acid, Protocatechuic acid, p-Coumaric acid, o-Coumaric acid, Vanillic acid, Gallic acid, Caffeic acid, Ferulic acid, Syringic acid, Sinapic acid, Ellagic acid and Chlorogenic acid. Sarkar and oba (2018) identified twenty-five phenolic and favonoid compounds including protocatechuic acid, salicylic acid, gentisic acid, gallic acid, β-resorcylic acid, vanillicacid, phydroxybenzoic acid, chlorogenic acid, ellagic acid, syringic acid, ferulic acid, kaempferol,m-coumaric acid, trans-cinnamic acid, quercetin, p-coumaric acid, apigenin, cafeic acid, rutin, sinapic acid, isoquercetin, naringenin, myricetin, catechin, and hyperoside in amaranth gangeticus.

The six compounds including Eugenyl O-β- Dglucopyranoside (citrusin C) were isolated from the leaves of C. argentea which shows Tyrosinase inhibitory and superoxide scavenging activity. The six compounds isolated from the leaves of C. argentea showed good skin depigmentation effect. A phenolic glycoside, 4-O- β-Dglucopyranosyl-2-hydroxy-6-methoxyacetophenone along with ten known compounds were isolated from the plant C. argentea (Sawabe et al., 2002).Moreover, a specific, simple, rapid and sensitive high performance liquid chromatography with tandem mass spectrometry method was developed and validated for determination of 13 components of C. communis Linn., which included orientin, iso-orientin, vitexin, isovitexin, rutin, apigenin, protocatechuate, vanillic acid, caffeic acid, ferulic acid, luteolin, quercetin and isorhamnetin (Zhang et al., 2018).

Flavonoids (ug g-1 FW): Flavonoids, a group of natural substances with variable phenolic structures, are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine. These natural products are well known for their beneficial effects on health and efforts are being made to isolate the ingredients so called flavonoids. Flavonoids are now considered as an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is attributed to their anti-oxidative, antiinflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function. In the present investigation, fifteen flavonoids compounds were identified by comparing their retention times and mass spectra with respective standards. The perusal of mean values indicated that flavonoids viz., Umbelliferone, Apigenin, Galangin, Naringenin, Kaemperol, Hesperetin, Quercetin, Epigallocatechin, Myricetin, Rutin, Luteolin, Fisetin, Eriodictyol, Catechin, Epicatechin. Subban et al., 2008, noticed two new flavonoids named castilliferol 1 and castillicetin 2, as well as a known compound, isochlorogenic acid 3, were isolated from the whole plant of Centella asiatica. Palmela reported total flavonoid content ranged from 8.91 to 9.93 mg CE/100g, with highest value observed for Amaranthus cruentus followed by A. hybridus and A. hypochondriacus then A. Hybrid and A. Caudatus. The result for total flavonoid obtained in this study is lower than 13.4 to 14.3 mg CE/100g obtained for A. hypochondriacus and 17.7 mg/100g obtained for oat. It was reported that there were no quantifiable amount of flavonoids in Amaranthus caudatus seeds, only traces of quercetin was found. Low levels of quercetin glycoside, rutin  $(4.0-10.2 \text{ µg/g})$  was detected in A. hypochondriacus seeds.

Vitamins: Vitamins are organic substances found in foods necessary for small quantities for the body's normal functioning. There are thirteen known vitamins in human nutrition divided into two groups according to their solubility. Water-soluble vitamins are composed of B group vitamins (thiamine, riboflavin, niacin, vitamin  $B_6$ , pantothenic acid, biotin, folate, and vitamin  $B_{12}$ ) and vitamin C. Water-soluble vitamins act as coenzymes and are involved in many biochemical reactions such as energy metabolism, amino acid metabolism, biosynthesis of amino acids, fatty acids, and pentose sugars, DNA synthesis, and transferring one-carbon unit and serve as antioxidants in many biochemical reactions. Fat-soluble vitamins of Vitamin A, D, E and K structurally

resembles partially cyclised isoprenoid polymers and are soluble mainly in lipids or oils and thus called fat-soluble vitamins. Absorption and transportation of these vitamins in the body is mainly associated with lipids in the intestine and stored in liver and adipose tissue and eliminated slowly from the body owing to their lipophilic character. High intake of fatsoluble vitamins may result in their accumulation in the body known as Hypervitaminosis due to the cause of delayed elimination rate. In the present investigation, eight water soluble (Niacin, Pyridoxin, Pantothenicacid, Biotin, Thiamine, Riboflavin, Folic acid, Cyanocobalamine) and five fat soluble vitamins (Vitamin D1, Vitamin D2, Tocopherol, Vitamin K2, Vitamin K1) were identified by comparing their retention times and mass spectra with respective standards. Marcardanate reported that the composition of carotenoids in five native Brazilian leafy vegetables was determined. The ranges of total carotenoid contents of Amaranthus viridis, Lepidium pseudodidymum, Xanthosoma spp., Sonchus oleraceus, and Portulaca oleracea were 347-468, 237-280, 225-361, 149-334 and 71-109 pg  $g^{-1}$ , with lutein and pcarotene predominating. The mean p-carotene contents (pg  $g^{-1}$ ) and vitamin A values (retinol equivalents RE  $g^{-1}$ ) were 110 and 18.4, 84.6 and 14.1, 67.3 and 11.2, 62.9 and 10.5, 29.8 and 4.99, respectively. The experimental result showed that the amount of Vitamin C in ten wild edible plants ranged from  $0.76 \pm 0.03 - 15.35 \pm 0.11$  mg/100 g. The quantity of Vitamin C was found highest in the leaves of *B. purpurea* (95.54  $\pm$  3.33 mg/100 g) followed by in the leaves of D. esculentum (5.41  $\pm$ 0.03 mg/100 g). The Vitamin C content in these wild edible plants is very much comparable with some common fruits and vegetables such as *Solanum tuberosum*  $(17.04 \pm 1.18 \text{ mg}/100$ g), Allium sativum (13.06  $\pm$  1.10 mg/100 g), and Daucus carota sativus  $(2.55 \pm 0.72 \text{ mg}/100 \text{ g})$ . Vitamin C was not detected in other plants under investigation (Seal et al., 2018). The HPLC chromatogram showed the presence of different water soluble vitamins in the plant extract. Maximum amount of vitamin B5 (pantothenic acid) is noted M. pumilus (5.632 mg/100g) (Datta et al., 2018).

### **CONCLUSION**

- The results of the study on the profiles of individual phenolic acids showed that the following acids were present: t-Cinnamic acid, p-hydroxy benzoic acid, 3 hydroxy benzoic acid, salicylic acid, protocatechuic acid, p-hydroxy benzoic acid, o-hydroxy benzoic acid, vanillic acid, felic acid, gentisic acid, and so on.
- The study on individual flavonoids profile revealed the contents of Catechin, Apigenin, Fisetin, Naringenin, Eriodictyol, Myricetin, Epicatechin, Galangin, Kaemperol, Luteolin, Hesperetin, Quercetin, Rutin respectively.
- Water soluble vitamin profiling in selected underutilized leafy vegetables revealed highest contents of Pantothenicacid, Thiamine, Niacin, Pyridoxin, Riboflavin, Folic acid, Bioti, Cyanocobalamine respectively. Analogously, individual fat-soluble vitamin profiling in underutilized leafy vegetables revealed highest contents of Vitamin K2, Vitamin D1, Vitamin D2, Tocopherol, Vitamin K1 respectively

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