The Journal of the Science of Food and Agriculture, 2018, 2(1), 7-13
https://www.hillpublisher.com/Journals/jsfa/

Studies of nutritional properties and antioxidant potential in green leafy vegetables

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How to cite this paper: Yadav, R.K., Tomar, B.S., Pachauri, N. & Jain, V. (2018) Studies of nutritional properties and antioxidant po-tential in green leafy vegetables. *The Journal of the Science of Food and Agriculture, 2(1)*, 7-13.
http://dx.doi.org/10.26855/jsfa.2018.01.001

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Abstract

BACKBROUND: To estimate the nutritional and antioxidant potential of green leafy vegetables (GLV's), an investigation was carried out with ten GLV's, namely Fenugreek (Trigonella foenum graecum L.), amaranth (Amaranthus tricolor), spinach beet or palak (Beta vulgaris var. bengalensis), spinach (Spinacia oleracea), chenopod or bathua (Chenopodium album), sarson sag (Brassica juncea var. rapa), lettuce (Lactuca sativa), Swiss chard (Beta vulgaris spp. cicla) and Indian spinach or poi (Basella spp.) involving their most commonly grown and popular varieties/landraces in India to determine their nutritional and antioxidant composition.
RESULT: Among leafy vegetables ascorbic acid was found maximum in amaranth variety Pusa Kiran (67mg/100g) followed by Chenopod cultivar Bathua Local (60.6 mg/100 g fw.). Significantly high level of total carotenoids and beta carotene was recorded in amaranth cultivar Pusa Lal Chaulai (69.4 and 7.8 mg/100 g fw. respectively) & Pusa Kiran (59.0 and 6.2mg/100g fw respectively). CUPRAC was recorded maximum in chenopod cultivar Bathua Local (32.7 µ moltrolax/g) followed by Pusa Bathua-1 (23.0 µ moltrolax/g), however Swiss Chard Local (4.2 µ mol ascorbic acid/ g f.w.) recorded maximum FRAP value. Amaranth cultivar Pusa Lal Chaulai recorded maximum poly phenol content among GLV's (1134 µg gallic acid equivalent (GAE)/ g) , while palak cultivar Pusa Bharati recorded minimum (154 µg GAE/ g). As for as dry matter content was concerned Swiss chard recorded significantly higher dry matter (22.9 %) than other GLV's. Several genotypes were identified as rich source of mineral viz. Pusa Lal Chaulai for iron content (11.2 mg/100g), Local Chenopod for calcium (271.3mg/100g), spinach Sel-VSPS for zinc (12.6 mg/100g), while for potassium chenopod Bathua Sel-2 (421.1mg/100g).
CONCLUSION: The antioxidant capacity of different leafy vegetables were in order of amaranth > chenopod > spinach > palak > fenugreek > sarson sag. These findings of this study will be helpful in tackling the problem of malnutrition by creating awareness among the consumers about the importance of leafy vegetables and development of nutritionally rich genotypes by the breeders.

Keywords

Green leafy vegetables (GLV's), total carotenoids, ascorbic acid, phenolic content, nutritional content and antioxidant properties.

1. Introduction

In India and other developing countries a large portion of the population are suffering from malnutrition especially pregnant women and children below the age of 5 are suffering from various health problem due to less intake of nutrients especially vitamin A and mineral like iron and calcium, since wheat and rice are the principal food crops. The national Monitoring Bureau surveys all show that intake of green leafy vegetables is <30% of recommended daily allowance almost uniformly across India. Green leafy vegetables (GLV's) are rich source of several health promoting vitamins such as beta carotene, ascorbic acid and folic acid as well as contains minerals such as iron and calcium. The bioavailability of micronutrients from the meals based on green leafy vegetables is considered higher than those based on cereal and legumes [2]. They also suited for their cultivation in tropical, sub-tropical and temperate climates and grown all the year round. Studies by FAO/WHO has also suggested that daily intake of 400 g fruits and vegetables helps in prevention of chronic diseases such as heart disease, cancer, diabetes and obesity, as well as for prevention of alleviation of several micronutrients deficiencies especially in less developed countries [4]. As GLV's have long been recognized most abundant and cheap sources of vitamins and minerals [1,15] .The use of antioxidants vitamins like ascorbic acids, are important in human food, subsequently they are also functioning as an anticancer agent. Though many leafy vegetables are grown in India, but few of them especially, amaranth, fenugreek, palak and spinach have attained commercial status and its cultivation is wide spread. Because of their low production cost and high yielding capacity, GLV's are considered to be one of the cheapest vegetables in the market and it could be rightly described as 'poor man's vegetables'. Being a cheap source of nutrients, utilization of GLV's in diet will be helpful in alleviation of malnutrition from the society.

2. Materials and methods

Experimental material and growing conditions

The present investigation was carried out at ICAR-Indian Agricultural Research Institute, New Delhi situated at 28.08^0 N and 77.12^0 E with an altitude of 228.61 m. The climate is subtropical and semi-arid. Popular genotypes of 10 Green Leafy Vegetables (GLV's) were studied. Details of crop and its species and genotypes/varieties used in this study are given in Table 1. All the genotypes were grown at research farm, Division of Vegetable Science, IARI, New Delhi during November-April 2013-14 and 2014-15. The standard recommended practices of growing Green Leafy Vegetables were followed.

Sample preparation for determination of various biochemical parameters

The tender and marketable leaf samples from each genotype were taken at first harvest. 5 g fresh leaf samples were washed under distilled water followed by double distilled water. They were drained completely, dried over filter paper and homogenized with motor and pestle in 30 ml ethanol (100%) and centrifuged at 10,000 rpm for 30 min at 4^0C. Supernatants of different samples were taken out. All analyses were carried out triplicate.

Determination of total carotene, ascorbic acid, poly phenols, and antioxidant activities

Total carotene was determined by using acetone and petroleum ether as extracting solvents, ascorbic acid was estimated titrimetrically using 2, 6-dichlorophenolindophenol and dry matter was estimated by drying 100 g fresh samples in hot air oven at 50˚C for 24 hours [14]. Total polyphenols content in ethanol extracts was determined with Folin-Ciocalteaureagent using gallic acid as a standard [17]. Cupric ion reducing antioxidant capacity (CUPRAC) was analysed utilizing the copper (II)-neocuproine [Cu (II)-Nc] reagent as the chromogenic oxidizing agent [5]. Ferric ion Reducing Antioxidant Power (FRAP) assay was analysed by standard method [6].

Extraction of β-carotene from total carotenoids

Solution having carotenoids was passed through column containing Hyflosupercel and magnesium oxide (3:1, v/v) followed by petroleum benzene and acetone. Separation took place with two different bands one is orange in colour i.e. β carotene.

Sample preparation for UPCC

The orange color β carotene collected through column chromatography was then dried using vacuum dryer and is then mixed with 3ml Hexane. The sample was then filtered through 0.45 µm Millipore filter. The injection volume of 1 µl was used for the run.

Sample preparation for minerals analysis

Edible portion of different leafy vegetables were washed and cleaned then oven dried at 50˚C for 24 hours. The dried sample was then pulverized into fine powder in a mortar pestle. About 0.5g dried sample was extracted with 10 ml of nitric acid at different temperature stages by using digestion unit. Once cooled, the solution was diluted to 50mL distilled water then volume was made to 100ml with distilled water. No further sample preparation was required. 10ml solution was taken from 100mL volume for MP-AES (microwave plasma- atomic emission spectrometer). The injection volume of 1µl was used for the run.

Statistical analysis

The average value of 3 replications was used for statistical analysis. Differences between samples were tested by analysis of variance (ANOVA) to assess differences group means. P values ≤ 0.5 were considered significant.

Table 1. Green leafy vegetables, their botanical names and cultivar used in this study.

S.No.	Genotype/ common name	Botanical name	Varity/land race
1.	Fenugreek or methi	*Trigonella foenum graecum*	Pusa Early Bunching (PEB), Pant Ragini
2.	Kasuri methi	*Trigonella corniculata*	Pusa Kasuri
3.	Amaranth	*Amaranthus tricolor*	Pusa Lal Chaulai (PLC) (red leaves), Pusa Kiran (green leaves)
4.	Palak	*Beta vulgaris var. bengalensis*	Pusa Bharati, All Green, Pusa Harit
5.	Spinach	*Spinacia oleracea*	PS-1 (prickly seeded), Sel-vs, Sel-VSPS, Varginia Savoy ((round, smooth seeded)
6.	Chenopod	*Chenopodium album*	Pusa Bathua-1(red leaves), Bathua Sel-2, Chenopod Sel-3), Bathua Local (green leaves)
7.	Sarson sag	*Brassica juncea var. rapa*	Pusa Sarson-1
8.	Lettuce	*Lactuca sativa*	Great Lakes
9.	Swiss chard	*Beta vulgaris* var. *cicla*	Local Chard
10.	Indian spinach or Poi	*Basella alba*	Local Poi Green
11.	Indian spinach or Poi	*Basella rubra*	Local Poi Red

3. Results and discussion

Significant variation was observed amongst different Green leafy vegetables (Table 2) from nutritional point of view. Ascorbic acid content was found to be significantly higher in amaranth cultivar Pusa Kiran (67 mg per 100 g) followed by chenopod cultivar Bathua Local (60.6 mg/100g) and spinach cultivar PS-1 (51 mg/100g) than all other GLV's. The lowest value of ascorbic acid was found in *Basella* cultivar Local (1.25 mg per 100 g). The cultivar with high values of ascorbic acid-fleshed can be utilized for nutraceutical industries, as ascorbic acid works as an electron donor, and therefore a reducing agent or antioxidant and high intakes of ascorbic acid correlate with reduced gastric cancer risk [11]. Green amaranth contains moderately high value of vitamin C [12,18]. The ascorbic acid content of the green leafy vegetables (GLV) ranging between 15.18 mg/100 g for *Centella asiatica* to 101.36 mg per 100 g for *Trigonella foenum graecum* [8].

It was also evident from the (Table 2) that total carotenoids contents varied significantly in GLV's ($P \geq 0.5$). The total carotenoids contents (TCC) was measured maximum in Bathua Sel-2 followed by amaranth cultivar Pusa Lal Chaulai (69.4 mg per 100 g fw.) and Pusa Kiran (59.0 mg per 100 g), among other GLV's Swiss chard cultivar Local Chard (45.0 mg/100g) and chenopod cultivar Pusa Bathua-1 (40.9 mg/100g) recorded high value of total carotenoids, which was significantly lower than amaranth. Local Red and Local Green *Basella* cultivar recorded equal and lowest value of TCC (10.2 mg per 100 g). On the basis results, leafy vegetables, namely amaranth, Swiss chard and *Chenopodium* were found to be the potential source for carotenoids. Absorption of β-carotene from green leafy vegetables was reported to be much higher, ranging from 50 to 99%. While from other sources, such as carrots and papayas, lower absorption of β-carotene has been reported [10]. Results revealed that maximum β-carotene was recorded in Bathua Sel-2 (8.5 mg/100g) followed by amaranth cultivar Pusa Lal Chaulai and Pusa Kiran (7.8 and 6.5 mg/100g respectively). While high beta carotene was also recorded in fenugreek, spinach, Swiss chard and cheopods.

Table 2. Ascorbic acid, total carotenoids and β-carrotene content in different green leafy vegetables.

Variety	Ascorbic acid (mg/ 100g)	Total Carotenoid (mg/100g)	β-carrotene (mg/100g)
PEB	7.56	30.9	2.5
Pusa Kasuri	5.50	31.3	2.9
PLC	11.3	69.4	7.8
Pusa Kiran	67	59.0	6.5
Pusa Bharati	27	29.7	3.0
All Green	26	22.6	2.7
PS-1	51	32.0	3.9
Varginia Savoy	25.2	35.6	4.9
Pusa Bathua-1	20.67	40.9	4.4
Bathua Sel-2	50	91	8.5
Bathua Local	60.6	44.7	4.5
Pusa Sarson-1	21.25	22.9	1.0
Great Lakes	3.75	22.0	1.5
Local Chard	2.5	45.0	5.9
Local Poi Red	1.25	10.2	0.9
Local Poi Green	1.25	10.2	1.9
CV (%)	11.50	6.50	8.50
CD (5%)	5.60	3.32	0.56

The antioxidant activity of phenolic compound is mainly due to their redox properties, which allows them to act as reducing agents. They act as hydrogen donors, singlet oxygen quencher, heavy metal chelators and hydroxyl radical quenchers [9]. It is revealed in the Table 3 that GLV's differed significantly with respect to their total phenolic contents ($P \geq 0.5$). Significantly higher total phenolic content (1134 µg gallic acid equivalent (GAE) per g fw.) was recorded in amaranth cultivar Pusa Lal Chaulai followed by Pusa Kiran (941 µg GAE per g fw.), which differed highly significantly from other GLV's under study i.e. chenopods Local (750 µg GAE/g fw.) and Pusa Bathua-1 (647 µg GAE/g fw.). The lowest value of polyphenol was recorded in palak cultivar Pusa Bharati (154 µg GAE per g fw.). The total phenolic content of *Amaranth* species to be 158.3 mg of tannic acid per 100 g of fresh vegetable [9]. Variation for total phenolic content is reported in different genotypes of amaranth and *Chenopodium* [18, 19]. Variation in total phenolic contents (5–69.5 mg of tannic acid/g of extract) was also reported earlier in leafy vegetables [16]. Therefore, it was concluded that the total polyphenol content of leafy vegetables varies widely depending on the variety.

Increase in antioxidant effects with increase in the reducing power of GLV's indicating that the antioxidant properties are concomitant with the change of reducing power of leafy vegetables. Among all the GLV's maximum antioxidant activity by CUPRAC method was recorded in *Chenopodium* cultivar Bathua Local (32.7 µmoltrolax per g), which was significantly higher than other GLV's (Table 3). Pusa Sag-1 (5.1µmoltrolax per g) variety of Sarson sag (leafy mustard) and lettuce cultivar Great Lakes (5.9 µmoltrolax per g) were found to be low in antioxidant content, while the minimum value of antioxidant was recorded in palak cultivar All Green (4.4 µmoltrolax per g). Similar study was done in *Amaranthus cruentus* (3.94 µ MTE/ g of dry weight) by CUPRAC method [7]. Similarly among different GLV's, high antioxidant activity by FRAP method was found in Swiss chard cultivar Local Chard (4.2 µ mol ascorbic acid equivalent (AAE) per g fw.), spinach cultivar PS-1 (3.1 µ mol AAE per g fw.) and *Chenopodium* genotype Bathua Local (2.5 µ mol AAE per g fw.). By FRAP method also antioxidant activity was reported high in *Amaranthus cruentus* v. Aztec (3.37 mM Fe^{+2} Kg^{-1} DW) [13]. The results envisaged that intensely coloured genotypes of different GLV's (chenopods, Swiss chard and spinach) recorded high antioxidant activities as compared to their green counterpart. Therefore, genotypes with coloured edible parts i.e. leaf and stem should be promoted for consumption in GLV's.

Table 3. Cuprac, Frap, total polyphenols and dry matter contents of different green leafy vegetables. (Values are mean ±SD of 3 determinations)

S.No.	Genotype/ common name	Varity/type	CUPRAC (μ mol trolax /g)	FRAP (μ mol Ascorbic Acid/ g fw.)	Phenols (μg Gallic Acid Equivalent/g fw.)	Dry matter (%)
1	Methi	P EB	12.65±0.66	0.87±0.21	302.80±0.72	12.40±0.46
2	Kasuri methi	Pusa Kasuri	10.98±0.59	0.36±0.18	417.90±0.85	11.60±0.26
3	Amaranth	P LC	18.60±0.62	2.27±0.21	1133.73±0.64	8.97±0.45
4	Amaranth	Pusa Kiran	16.30±0.70	1.40±0.36	941.57±0.51	9.53±0.15
5	Palak	Pusa Bharati	8.50±0.50	1.50±0.30	154.43±0.51	6.13±0.32
6	Palak	All Green	4.43±0.35	0.67±0.25	301.60±0.53	6.67±0.25
7	Spinach	PS-1	21.83±0.76	3.17±0.31	397.00±1.00	6.83±0.31
8	Spinach	Varginia Savoy	17.78±0.35	2.30±0.46	225.73±0.64	7.83±0.31
9	Chenopod	Pusa Bathua-1	23.13±0.32	1.70±0.26	647.03±0.95	10.40±0.50
10	Chenopod	Bathua Local	32.73±0.35	2.60±0.26	750.47±0.50	16.53±0.35
11	Sarson sag	Pusa Sarson-1	5.12±0.35	0.17±0.02	234.00±1.00	13.13±0.32
12	Lettuce	Great Lakes	5.86±0.41	0.06±0.03	202.47±0.50	8.50±0.40
13	Swiss chard	Local Chard	16.00±1.00	4.27±0.21	201.30±1.47	22.80±0.26
14	Indian spinach or Poi	Local Poi Red	9.72±0.20	0.34±0.03	311.67±1.53	12.60±0.26
15	Indian spinach or Poi	Local Poi Green	9.51±0.36	0.34±0.04	475.00±1.00	12.23±0.25

It was evident from Table 3 that red leaf colour amaranth had higher poly phenols and antioxidant activity (CUPRAC and FRAP) than green. The higher amount of pigments, total polyphenol and antioxidant activity was reported in red leaf cultivar of amaranth as compared to green leaf cultivar when exposed to various levels of shade [3]. Similar observation was recorded by phosphomolybdenum method in antioxidant properties of GLV's and the order of antioxidant properties of different vegetables were found as *Murraya koenigii>Trigonella foenum graecum>Amaranthus spp.>Centella asiatica* [8].

Swiss chard cultivar Local Chard (22.8%) recorded significantly high value of dry matter (%) followed by *Chenopodium* cultivar Bathua Local (16.53%), showed that high processing recovery may be obtained from these.

Mineral content in different leafy vegetables varied from genotype to genotype within a species. The genotypes having high value for a particular mineral were only used for graphical representation (Figure 1 and 2). Iron an important mineral in the diet especially for pregnant and nursing women was recorded very high in Pusa Lal Chaulai (11.2 mg/100g) followed by Chenopod Sel-2 and Chenopod Sel-3 (7.6 mg/100g each). Zinc another important mineral was estimated maximum in spinach Sel-VSPS and Local chenopod (1.3 mg/100g) followed by Virginia Savoy & Chenopod Sel-2 (9.8 mg/100g). Aluminium content was also significantly high in Amaranth genotype Pusa Lal Chaulai (12.6 mg/100g) than other leafy vegetables. Manganese content showed very less variation within and between different leafy vegetables (Figure 1).

Calcium an important mineral for growing children and old people was recorded maximum in Chenopodium genotype Local Chenopod (271.3 mg/100g) followed by Chenopod Sel-2 (265mg/100g). High content of sodium was recorded in amaranth cultivar Pusa Kiran followed by Pusa Kirti and scented Fenugreek cultivar Pusa Kasuri, while potassium content was recorded maximum in Chenopod Sel-2 (421 mg/100g) followed by Local Chenopod (412 mg/100g). Potassium content varied significantly amongst different leafy vegetables. High potassium content was also recorded in Fenugreek and spinach. Magnesium content ranged from 51 mg/100g (Pusa Harit) to 195mg/100g (Local Chenopod) (Figure 2).

The finding revealed that leafy vegetables were rich source of iron, calcium, magnesium, sodium and potassium.

However, significant genotypic difference within a crop was noticed for mineral content. On overall basis, Chenopod was found rich in most of the mineral under study followed by amaranth, spinach and fenugreek. Analysis of various green leafy vegetables for 8 micronutrients recorded higher bioavailability of meals based on leafy vegetables than those based on cereal and legumes [2].

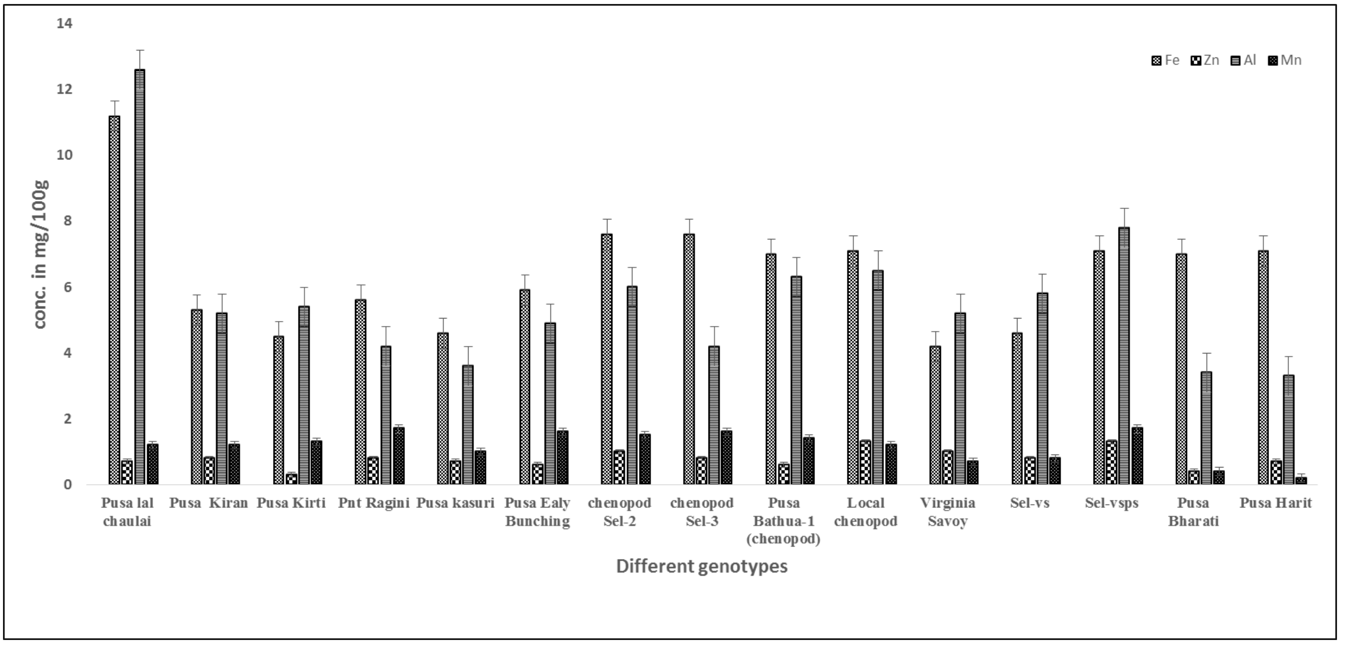

Figure 1: Iron (Fe), zinc (Zn), Aluminium and Manganese (Mn) (mg/100g) and total carotenoids (mg/100g) in different Indian Leafy vegetables ($p \leq 0.05$).

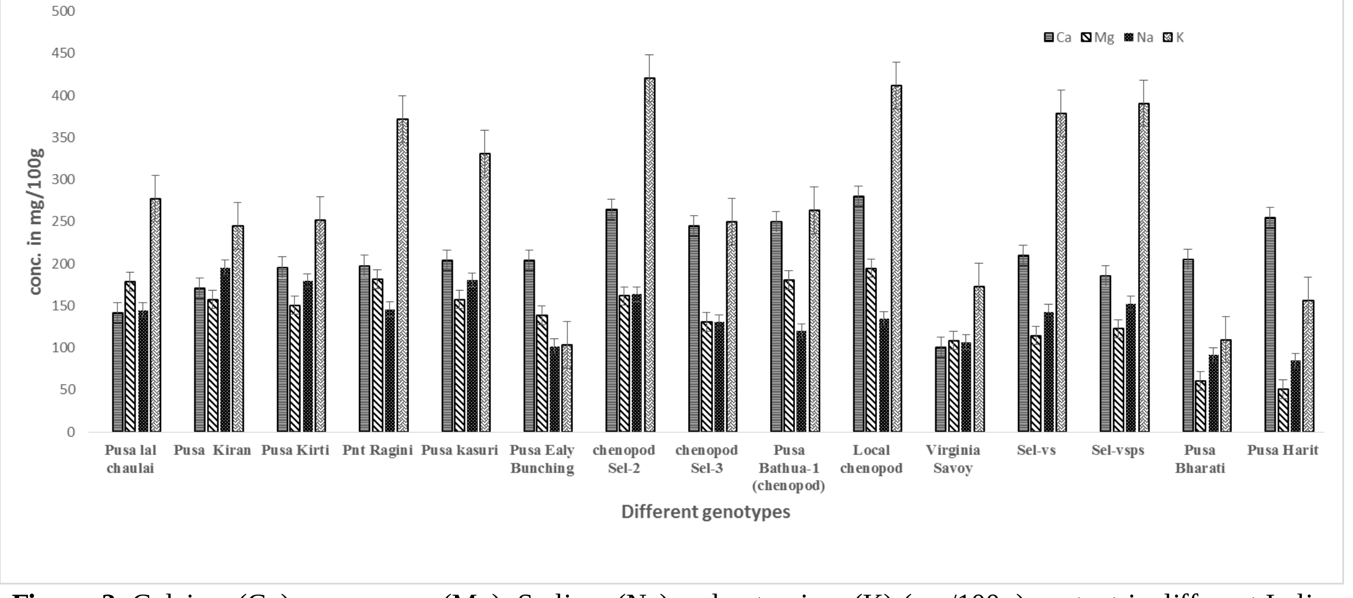

Figure 2: Calcium (Ca), manganese (Mg), Sodium (Na) and potassium (K) (mg/100g) content in different Indian Leafy vegetables ($p \leq 0.05$).

If the growers are interested to produce high quality leafy vegetables in regard to health benefits the varieties rich in various antioxidants should be cultivated. Therefore, the results suggested that Pusa Lal Chaulai (amaranth), Bathua Local and Bathua-2 (chenopod), PS-1, Sel-VSPS (spinach), Pusa Bharati (palak) and Swiss chard local may be popularised and made available to the consumers. Based on the findings it was concluded that the amaranth, chenopod, spinach had the high antioxidant activity and could be utilized for improving the efficiency of different nutraceutical and pharmacological products. The consumption of these leafy vegetables play an important role in preventing human diseases in which free radicals are involved such as cancer, cardiovascular diseases and aging. It was noticed that most of the Indian traditional leafy vegetables like, amaranth, *Chenopodium*, *Fenugreek*, *Basella* were rich in most of the nutrients like calcium, iron as compared to exotic vegetable spinach. They have potential as remedy to counter to food and nutritional insecurity as they are well adapted to local environment, less affected by pests, diseases and drought and are cultivated by resource poor farmers living in remote area.

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