

## Original Research Article

## Phyto-Chemicals, Antioxidants and Nutritional Constitutes of Some Non Conventional Green Leafy Vegetables Grown in sub Himalayas

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**Abstract:** There are many types of other non conventional green leafy vegetable which are grown and consumed locally but has not been explored nutritionally. These non conventional green leafy vegetables are not cultivated but they grow naturally near water and in the open space. The present study was conducted to evaluate the phyto-chemicals, anti oxidant and nutritional quality of the selected non conventional green leafy vegetables grown locally /wild in Himachal Pradesh using standard methods. Five non conventional green leafy vegetables i.e. *Bharase*, *Fafru*, *Khokhuya*, *Bichu butti* and *Chu-nali* were taken for the study. The parameters analysed were. Flavonoids, DPPH activity proximate constituents dietary fiber and mineral content. The results revealed that there was variation in the physical characteristics of the all non conventional green leafy vegetables. Nutritional quality evaluation revealed that the *Bichu butti* (*Utrica diocia*) was more nutritious as it contained 3.46 per cent protein, 11.38 per cent carbohydrates, 2.20 per cent ash and also 62.92kcal/100gm energy on fresh weight bases as compared to other non conventional green leafy vegetables. DPPH (57.07%) and Flavonoids (9.04%) were maximum in *Bichu butti* followed by *Chu-nali*. *Bharase* contained higher amount of macro and micro elements.

**Keyword:** Antinutrients, Dietary Fiber, Phytochemicals, Non Conventional green leafy vegetables, Calorific value, Minerals, DPPH.

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### INTRODUCTION

Vegetables are plants or parts of plants which are used as food and called as protective foods because these are rich in vitamins, minerals and antioxidant. These are those plants which are served raw or cooked as a part of main course of the meal. Indian, being bestowed with a variety of natural surrounding and varying climates and seasons has a number of edible green leafy vegetables some of which are grown and consumed locally. However, only a very small percentage of edible green leafy vegetables are being utilized for human consumption.

Green Leafy Vegetables have occupied a unique place in vegetables because of their colour, flavour and health benefits. They are rich source of vitamin such as  $\beta$ -carotene, ascorbic acid, folic acid and riboflavin as well as minerals such as iron, calcium, phosphorous. They also contain an immense variety of bioactive non nutritive health promoting compounds such as antioxidants and phytochemicals which provide health benefits beyond basic nutrition. Singh *et al.*,

2001 [1]. Hidden hunger or micronutrient deficiency is a major nutritional problem in developing countries, The major reason for nutritional deficiency is the increasing gap between food supply and population growth. To meet the nutritional requirement of the population it is important for diversification towards non-conventional nutritional crops which includes cereals, legumes, fruits and vegetables. The negligence of familiar crops is not due to their any inferior nutritional quality but due to lack of research resources in the place of origin and thus often scorned as “poor people’s plants”. But recently a resurgence of interest has developed in wild species because of their immense nutritional and medicinal values in diet.

Many leafy vegetables especially, amaranth, fenugreek, mustard leaves, chenopodium leaves and spinach has attained commercially status and its cultivation is wide spread. There are many types of other non conventional green leafy vegetable which are grown and consumed locally but has not been explored nutritionally. These non conventional green leafy

vegetables are not cultivated but they grow naturally near water and in the open space. The vegetables like *bharase*, *lungru*, *kachalu*, *chu-nali*, *khokhuya*, *bathua*, *bichu butti* etc are some of green leafy vegetables which are grown in sub Himalyan areas and had been consumed by local people but much work has not been done on their nutritional quality evaluation and photochemical profile. So the present study was planned to explore the biochemical and nutritional quality of these non conventional vegetable.

## MATERIALS AND METHODS

### Procurement and preparation of samples

Five non conventional green leafy vegetables i.e. *Chu-nali* (*Nasturtium officinale*) and *Bichu butti* (*Urtica dioica*) were collected from the near by forest where as *Fafru* (*Fagopyrum cymosum*), *Bharase* (*Fagopyrum esculentum*), *Khokhuya* (*Stellaria media*) were procured from the local farmers/ households growing these vegetables After sorting, the leaves were washed with distilled water, dehydrated under the sunlight, then these were ground separately to form fine powder with the help of stainless steel electric grinder. These were stored in properly labeled airtight glass containers at ambient temperature so as to prevent changes till further analysis. All the analysis were performed in triplicates. Fresh leaves were used for measuring the physical parameters like weight length and breadth.

Weight of ten fresh randomly selected leaves was measured in an electric balance whereas length and breadth of ten fresh leaves with the help standard scale for determination of size.

Water absorption capacity measured by the method described by Sosulski and Garratt [2]. Oil absorption measured by the method described Lin [3].

The nutrient constituents constituent's viz. moisture content, crude fat, crude fiber, ash sugars i.e. total sugars reducing and non reducing sugars is in the samples were analyzed by using standard methods AOAC [4].

Nitrogen was analyzed by Micro kjeldhal method and was multiplied with the factor of 6.25 for converting it in to crude protein AOAC2010 Non Protein Nitrogen (NPN) in samples was determined by the method of Pellet and Young [5]. Difference between non protein nitrogen and crude protein nitrogen gave the value for true protein nitrogen

Total carbohydrates were calculated by the following formula:

Total carbohydrate (%) = 100- (Moisture + crude ash + crude fiber + crude protein + crude fat)

Energy in the samples was determined by chromic oxide method of O'shea and Maguire [6].

Dietary fiber constituents include Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Lignins, Cellulose and Hemicellulose were estimated by the method as suggested by Van Soset and Wine [7]. The ascorbic acid, Beta carotenes and total chlorophyll in leaves sample was estimated by the method of Rangana [8]. For estimation of minerals diacid mixture was used for digestion and mineral viz calcium, Iron and zinc were determination by atomic absorption spectrophotometer (Perkin Elmer Analyst 400). Phosphorus was estimated Total Phenols were estimated by method of Makkar *et al.*, [9]. Flavonoids were estimated by method of Boham and Kocipia 1994. Antioxidant Activity was analysed by the methods of Miliukaus *et al.*, [10] and Khalaf *et al.*, [11].

### Statistical Analysis

Difference among chemical constituents of were statistically analyzed using statistical tools as completely randomized design at 5% level of significance ( $p \leq 0.05$ ).

### Functional properties and Physical Characteristics

Functional properties of food material describes that how the ingredients will behave during preparation, cooking and processing and how the finished food product will be affected in terms of appearance, taste, and feel. As far as the data functional properties of the leaves were concerned varietal differences were there in non conventional green leafy vegetables (table 1). Maximum water absorption capacity was in *Fafru* (7.10ml/g) and minimum was in *Bharase* (5.03ml/g) whereas, maximum oil absorption capacity was present in *Bharase* (4.10ml/g) and minimum was in *fafru* and *Bichu butti* (3.00ml/g). The higher oil absorption capacity of *Bharase* might be due to higher fibre constituent in *Bharase*. A significantly ( $p \leq 0.05$ ) higher water absorption capacity was observed for *Fafru* when it was compared with other counter parts whereas a non significant ( $p \leq 0.05$ ) difference was observed in the water absorption capacity of *chunali*, *khokhuya* and *bichu butti* when compared with each other, but they differ significantly ( $p \leq 0.05$ ) when compared with *Bharase*. Water absorption capacity of *Fafru* was higher which might have been due to the reason that it might have high hydration capacity as compared to other green leafy vegetable powder, also due to different chemical composition.

In physical parameter the colour of the *Bichu butti*, *Bharase* and *Chu-nali* was dark green whereas *Fafru* and *khokhuya* was light green in colour.

Shape of the non conventional green leafy vegetables viz. *Chu-nali* (*Nasturtium officinale*), *Fafru* (*Fagopyrum cymosum*), *Bharase* (*Fagopyrum esculentum*), *Khokhuya* (*Stellaria media*), *Bichu butti* (*Urtica dioica*) was different from each other. Maximum leaf weight was found in *Chu-nali* (19.16g)

and minimum was in Khokhuya (12.21g). The average length was highest in Bichu butti (5.11cm) and minimum was in Khokhuya (1.92cm). The variation in the physical properties of the leaves was due to varietal differences.

### Proximate Composition

The proximate composition of the non conventional green leafy vegetables of leaves showed that the moisture content was maximum in Chu-nali (87.59%) and whereas ash, carbohydrates and protein was maximum in the Bichu butti (2.20, 11.38 and 3.16%) whereas Khokhuya contain maximum amount of crude fiber (2.15%) and non significantly ( $p \leq 0.05$ ) higher ether extract was found in Fafru (0.63%). The values of proximate composition of the non conventional green leafy vegetables are on fresh weight bases. A significant ( $p \leq 0.05$ ) difference in moisture content in all the five non conventional green leafy vegetables, when compared with each other the variation in moisture content of the vegetables might have been due to different varieties of the vegetables and also due to agro climatic condition under which they have grown. *Chu-nali* has grown near water sources as a result it had significantly ( $p \leq 0.05$ ) higher moisture content as compared to other four leafy vegetables to [12] the moisture content found in *Utrica diocia* was 89 per cent respectively. Other worker [13] reported that moisture content of the *Nasturatum officinale* was 87.50 per cent. Similarly [14] reported that 83.72 percent moisture was in *Utrica urenus*. The results of the present investigation are at par with other worker however slight difference might have been due to the difference of varieties. The non significant ( $p \leq 0.05$ ) difference was observed in the crude fiber content of *Chu-nali* and *Fafru* when compared with each other. Variation of fiber content of five leafy vegetables might be due to the different varieties of non conventional green leafy vegetables.

### Nutritional Quality

Non Protein Nitrogen is the nitrogen present in food stuffs which does not contribute towards the proteins in the body. This includes the nitrogen from all nitrogenous substances other than proteins which includes uric acid, urea, creatinine, creatine, some peptides and free amino acids.

Data in Table-2 shows the non protein nitrogen content of non conventional green leafy vegetables. The values of NPN varied non significantly ( $p \leq 0.05$ ) in different non conventional leafy vegetables when compared with each other. The values ranged from 0.11 to 0.18 per cent respectively. The true protein content was significantly ( $p \leq 0.05$ ) higher in *Bichu butti* i.e. 1.99 per cent when compared with *Fafru*, *Bharase*, *Khokhuya* and *Chu-nali*. A non significant ( $p \leq 0.05$ ) difference was observed in the true protein content of *Bharase* and *Chu-nali*, *Fafru* and *khokhuya* when compared with each other. There was 0.85, 1.31, 1.89,

1.26 per cent true protein content was found in *chenopodium* leaves [15].

Carbohydrates are the main source of energy in the vegetarian diets. They are complex organic substances which are made up of carbon, hydrogen and oxygen. Hydrogen and Oxygen is generally in the ratio of 2:1. Carbohydrates are of two types available and non-available carbohydrates. Available carbohydrates are those which are digested in human system. They include starch and sugar whereas non-available carbohydrates are dietary fiber, pectin and gums which are not digested by the enzymes. Significantly ( $p \leq 0.05$ ) higher carbohydrate content was found in the *Bichu butti* when compared with other non conventional green leafy vegetables.

Maximum calorific value was found in *Bichu butti* (62.94kcal/100gm) and minimum was in *Chu-nali* (36.11 kcal/100gm). *Chu-nali* contain higher amount of  $\beta$ -carotene (1977.44 $\mu$ g/100gm) and *Fafru* was lower in  $\beta$ -carotene content (1477.70  $\mu$ g/100gm). *Chu-nali* contain higher amount of ascorbic acid (Vitamin C) in *Chu-nali* (49.39mg/100g) and minimum was in *Bichu butti* (29.48mg/100g).

Reducing sugar content in non conventional green leafy vegetables ranged from 0.13 to 0.19 per cent whereas non reducing sugar content ranged from 0.17 to 0.23 per cent. The maximum total sugar content was present in *Khokhuya* i.e. 0.42 per cent and minimum was in *Chu-nali* i.e. 0.19 per cent.

Dietary fiber is defined as that portion of food derived from plant cells, which is resistant to hydrolysis/ digestion by the elementary enzyme system in human beings. It includes different constituents like NDF, ADF, cellulose, Hemicellulose, gums, mucilages and pectin substances. Some degradation of dietary fiber takes place in the large intestine due to bacterial fermentation. Dietary fibre constituents i.e. NDF, ADF, and Lignin (46.70%, 35.10% and 24.32%) was maximum in *Bichu butti* whereas Cellulose was maximum in *Bharase* (16.52%) and minimum was in *Bichu butti* (11.60%). Highest Hemicellulose was found in *Khokhuya* (10.89%) and minimum was in *Bichu butti* (4.3%).

Mineral content of non conventional green leafy vegetables i.e. Iron, Zinc, Phosphorous and Calcium. Maximum amount of iron, zinc and calcium was found in *Bharase* i.e. 20.18mg/100gm, 3.4mg/100gm and 348mg/100gm whereas phosphorous was maximum in *Fafru* i.e. 835mg/100gm. *Chu-nali* contain higher amount of  $\beta$ -carotene (1977.44 $\mu$ g/100gm) and *Fafru* was lower in  $\beta$ -carotene content (1477.70  $\mu$ g/100gm). *Chu-nali* contain higher amount of ascorbic acid (Vitamin C) in *Chu-nali* (49.39mg/100g) and minimum was in *Bichu butti* (29.48mg/100g).

**Phytochemicals and Anti-oxidant activity**

Minimum flavonoid content was in Chu-nali (7.00%) and DPPH content was minimum in Bharase (47.96%). Chu-nali contain higher amount of Chlorophyll and total phenolic content (0.034g/l and 4.38mg/gTAE). A non significantly ( $p \leq 0.05$ ) was observed in chlorophyll content of all non conventional green leafy vegetables when compare with each other. Duma *et al.*, (2014) reported that the stinging nettles contained 2.070 mg/g of total chlorophyll content. Phenolic exhibit antioxidant potential due to their redox properties which allow them to act as reducing agents, hydrogen donators and single oxygen quenchers. Significantly ( $P < 0.05$ ) higher total phenol was found in the *Chu-nali* when compared with *Fafru*, *khokhuya* and *Bichu butti* but it differed non significantly ( $P < 0.05$ ) from the *Bharase*. A non significantly ( $P < 0.05$ ) difference was also observed in the total phenol content of *Khokhuya* and *Fafru*, *Khokhuya* and *Bichu butti* when compared with each other but significant difference ( $P \leq 0.05$ ) was observed as a total phenol content of *Bichu butti* and *Fafru* when compared with each other.

DPPH- It is a common abbreviation for the organic chemical compound 2,2-diphenyl-1-picrylhydrazyl. It is a dark coloured crystalline powder compared of stable free radical molecules. DPPH has two major applications, both in laboratory research: one is monitor of chemical reactions involving radicals, most notably it is a common antioxidant assay, and another is a standard of the position and intensity of electron paramagnetic resonance signals.

Data in table depicts the DPPH activity of non conventional green leafy vegetables as is clear from the table that *Bichhu butti* had the maximum DPPH activity that is 57.07% followed by *Chu-nali* i.e 54.69% and *Fafru* 52.39%, *Khokhuya* i.e 50.84% and *Bharase* 47.96%. A significant difference ( $p \leq 0.05$ ) was observed in DPPH content of all the non conventional green leafy vegetables when compared with each other. According to [16] the antioxidant activity in nettles leaves was 66.60 per cent. Slightly variation might have been due to the difference in varieties.

**Table-1: Physical characteristic non conventional Green leafy vegetables**

| Parameter    | FAFRU                         | BHARASE                      | BICHU BUTTI  | CHU-NALI                      | KHOKHUYA                      | CD ( $p \leq 0.05$ ) |
|--------------|-------------------------------|------------------------------|--|-------------------------------|-------------------------------|----------------------|
| Colour       | Light Green                   | Dark green                   | Dark green   | Dark Green                    | Light Green                   | -                    |
| Shape        | Heart shape, with pointed end | Heart shape with pointed end | Very Fine, thorn on the surface, Tapered and Heart shape | Lenceolate having pointed end | Oval shape, small or no point | -                    |
| Weight (g)*  | 18.86                         | 18.61                        | 16.54  | 19.16                         | 12.21                         | 0.32                 |
| Length (cm)* | 4.45                          | 4.56                         | 5.11   | 4.54                          | 1.92                          | 0.36                 |
| Width (cm)*  | 4.24                          | 5.19                         | 4.71   | 1.67                          | 1.41                          | 0.34                 |
| L/W ratio*   | 1.04                          | 0.87                         | 1.08   | 2.72                          | 1.35                          | 0.24                 |

\*On fresh weight bases

**Table-2: Proximate composition of the non conventional Green leafy vegetables**

| Parameter          | BHARASE           | CHU-NALI         | FAFRU            | BICHU BUTTI      | KHOKHUYA         | CD ( $p \leq 0.05$ ) |
|--------------------|-------------------|------------------|------------------|------------------|------------------|----------------------|
| Moisture (%)       | 86.11<br>(13.88)* | 87.59<br>(12.71) | 86.85<br>(13.18) | 81.64<br>(18.36) | 87.41<br>(12.95) | 0.13                 |
| Ash (%)            | 1.55<br>(11.18)   | 1.33<br>(10.36)  | 1.80<br>(13.73)  | 2.20<br>(11.97)  | 2.05<br>(11.56)  | 0.12                 |
| Crude fiber (%)    | 1.83<br>(12.97)   | 0.98<br>(7.71)   | 0.88<br>(6.60)   | 1.08<br>(5.88)   | 2.15<br>(13.23)  | 0.20                 |
| Ether extract (%)  | 0.59<br>(4.29)    | 0.48<br>(3.78)   | 0.63<br>(4.83)   | 0.54<br>(2.97)   | 0.35<br>(2.33)   | NS                   |
| Crude protein (%)  | 1.71<br>(12.35)   | 1.31<br>(10.35)  | 2.20<br>(16.80)  | 3.16<br>(17.19)  | 2.21<br>(14.55)  | 0.21                 |
| Carbohydrate (g)   | 8.19<br>(45.31)   | 9.57<br>(55.05)  | 7.64<br>(44.88)  | 11.38<br>(43.61) | 5.92<br>(45.38)  | 0.98                 |
| NPN (%)            | 0.11              | 0.14             | 0.16             | 0.18             | 0.15             | 0.10                 |
| True Protein (%)   | 1.24              | 0.79             | 1.19             | 1.99             | 0.62             | 0.20                 |
| Energy (Kcal/100g) | 44.98             | 46.65            | 49.89            | 62.94            | 36.11            | 0.43                 |

**Table-3: Sugar and Dietary fiber constituents of the non conventional Green leafy vegetables**

| Parameter               | FAFRU | BHARASE | KHOKHUYA | BICHU BUTTI | CHU-NALI | CD (p≤0.05) |
|-------------------------|-------|---------|----------|-------------|----------|-------------|
| Reducing Sugars (%)     | 0.15  | 0.13    | 0.19     | 0.14        | 0.11     | NS          |
| Non reducing sugars (%) | 0.22  | 0.19    | 0.23     | 0.18        | 0.17     | NS          |
| Total Sugars (%)        | 0.36  | 0.33    | 0.42     | 0.35        | 0.29     | 0.10        |
| NDF (%)                 | 40.89 | 35.76   | 45.37    | 46.70       | 39.74    | 0.13        |
| ADF (%)                 | 26.00 | 19.24   | 33.96    | 35.10       | 24.14    | 0.13        |
| Lignin (%)              | 16.99 | 8.87    | 22.92    | 24.32       | 13.38    | 0.13        |
| Cellulose (%)           | 15.63 | 16.52   | 11.74    | 11.60       | 15.60    | 0.74        |
| Hemicellulose (%)       | 8.88  | 10.37   | 10.89    | 10.78       | 10.76    | 0.13        |

**Table-4: Macro and Micro elements β- carotene Vitamin C, Phytochemicals, antioxidant and DPPH activity of non conventional green leafy vegetables**

| Parameter               | FAFRU   | BHRASE  | KHOKHUYA | BICHU BUTTI | CHUNALI | CD (p≤0.05) |
|-------------------------|---------|---------|----------|-------------|---------|-------------|
| Iron (mg/100gm)         | 15.6    | 20.18   | 10.11    | 19.2        | 5.8     | 0.19        |
| Zinc (mg/100gm)         | 2.9     | 3.4     | 2.8      | 1.9         | 2.4     | 0.53        |
| Phosphorus (mg/100gm)   | 835     | 825     | 545      | 620         | 750     | 0.23        |
| Calcium (mg/100gm)      | 318     | 348     | 285      | 301         | 250     | 0.31        |
| Ascorbic Acid (mg/100g) | 44.88   | 40.24   | 35.86    | 29.48       | 39.39   | 0.10        |
| β- carotene (µg/100g)   | 1477.70 | 1727.57 | 1722.61  | 1535.61     | 1977.44 | 0.25        |
| Flavonoids (%)          | 7.17    | 8.19    | 7.05     | 9.04        | 7.00    | 0.36        |
| Chlorophyll (g/l)       | 0.027   | 0.030   | 0.019    | 0.024       | 0.034   | 0.813       |
| Total Phenol (mg/gTAE)  | 3.15    | 4.13    | 2.72     | 2.08        | 4.38    | 0.98        |
| DPPH (%)                | 52.39   | 47.96   | 50.84    | 57.07       | 54.69   | 0.40        |

## CONCLUSION

Non conventional green leafy vegetables are rich in macro and micro elements but low in calorific value, sugars and carbohydrates. These are also rich sources of phytochemicals maximum flavonoid and antioxidant activity. Consumption of these non conventional green leafy vegetables should be encouraged as these are grown naturally and free from pesticides and insecticides.

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