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Proximate, mineral composition and antioxidant properties of some wild leafy vegetables

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We analysed five wild leafy vegetables (*Amaranthus viridis, Chenopodium album, Diplazium esculentum, Nasturtium officinale* and *Urtica dioica*) of Sikkim for proximate and mineral composition. Wild leafy vegetables (WLV) of Sikkim are found nutritionally rich in terms of calorific value, fibre, protein and low fat which altogether indicate the potentiality of the WLVs as good source of non-conventional vegetables. WLVs were also analysed for antioxidant properties, total phenolic content and vitamin C content. All five WLVs were found to exhibit moderate antioxidant activity with variability in total phenolic content and vitamin C content. It firmly establishes rich nutritional efficiency of WLVs in the local diets.

Keywords: Wild leafy vegetables, antioxidant, proximate and mineral composition.

Introduction

The ethnic people have inherited rich traditional knowledge of surrounding plants used as food, fodder, fibres, woods, fuel, medicine, beverage, tannin, dye, gum, resin, cosmetics, crafts and religious ceremonies[1]. The nutritional value of traditional wild plants is higher than several known common vegetables and fruits[2]. In developing nations, numerous types of edible wild plants are exploited as sources of food hence provide an adequate level of nutrition to the inhabitants[3]. Wild edible plants are valuable sources of nutrients especially in rural areas where they contribute substantially to protein, minerals, vitamins, fibres and other nutrients[4]. Vegetables are known as excellent sources of natural antioxidants[5]. The importance of antioxidant constituents of plant material has also been established in the maintenance of health[6]. Many people are not aware of the nutritional value of wild edible plants and many regard them as inferior[7,8]. Intake of traditionally consumed wild edible species is nowadays receiving renewed attention, due to the recognition of their potential benefit for human health[9]. Ethnic people of Sikkim consume roots, tubers, rhizomes, leaves and fruits of more than 190 species of wild edible plants[10]. Some of them sell the edible wild fruits and vegetables in nearby local markets of Sikkim[11], which are in high demand among the local consumers[12]. The present paper is aimed to determine proximate and mineral composition and antioxidant properties of some wild leafy vegetables (WLV) of Sikkim.

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Materials and methods

Collection of samples

A total of 50 samples (10 each) of wild leafy vegetable (WLV) species (*Amaranthus viridis, Chenopodium album, Diplazium esculentum, Nasturtium officinale* and *Urtica dioica*) were collected from different natural habitats of Sikkim. Collected plant materials were placed in a sterile poly-bag to prevent loss of moisture during transportation to the laboratory. The leafy parts of these vegetables were washed, cut and shade dried at room temperature, and dried leaves were pulverized, packed in airtight sterile bottles, labelled and stored in a desiccator for further analyses.

Preparation of methanolic extracts

The dried materials were ground and extracted (20 g) successively with 200 ml methanol in a glass soxhlet extractor at 130^0C for 24–48 h. The extract was concentrated by using rotary evaporator. The extract was preserved in a desiccator till further use.

Proximate analysis

Moisture content of the WLVs was determined by drying 2.0–3.0 g of sample at 135 ± 1° C for 2 h to constant weight[13]. Fat content of the sample was determined by ether extraction using glass soxhlet[13]. Crude fibre was estimated by acid–base digestion with 1.25% H_2SO_4 and was expressed as percentage loss in weight on ignition[14]. Total nitrogen of the sample was determined following the method described in AOAC[13]. Protein content was determined by multiplying total nitrogen value with 6.25 i.e., protein (%) = total nitrogen (%) × 6.25[13].

Carbohydrate content of the samples was calculated by difference: 100 – (% protein + % fat + % ash)[15]. Food value of each sample was determined by multiplying the protein, fat and carbohydrate contents by the factors 4, 9 and 4, respectively, and adding all the multiplication values to get kcal per 100 g[16].

Minerals

Calcium, sodium and potassium were estimated in flame-photometer (Thermo Scientific Chemito, India) following the method of Ranganna[17]. For analysis of magnesium, zinc, copper and iron, triacid digestion method of Prasad and Bisht[18] was followed with modification. It was carried out using HNO_3: $HClO_4$: H_2SO_4 in the ratio 9:4:1. The collected leaves were dried in an oven at 60-70° C for 2-4 h. The dried leaf samples of each plant material were ground separately and 1g of ground sample was digested in 15 ml of tri-acid mixture, and the mineral elements were determined using Atomic Absorption Spectroscopy (Perkin-Elmer Analyst 200, USA).

Vitamin C

Vitamin C was estimated following the direct colorimetric determination method as mentioned by Ranganna[17] and based on measurement of the extent to which a 2,6-dichlorophenol-indophenol solution is decolorized by ascorbic acid in sample extracts and in standard ascorbic acid solutions. 10 g of sample was accurately weighed and blended with 2% HPO_3. The solution was filtered and 2-10 ml aliquot of extract of HPO_3 of the sample was titrated and the reading was taken within 15-20 sec. The red colour was measured at 518 nm. The absorbance against concentration was plotted. The concentration of ascorbic acid was noted from the standard curve and the ascorbic acid content in the samples was calculated with the following formula. AA mg /100g = Ascorbic acid content x volume made up to x 100 /ml of solution taken for estimation x 1000 x weight or volume of sample taken

Total Phenolic Content

Total soluble phenolic compounds present in extracts were determined with the folin-ciocalteu reagent[19] with modification. Calibration curve was prepared by mixing methanolic solution of gallic acid with 1 ml folin–ciocalteu reagent and sodium carbonate (2%). Absorbance was measured at 760 nm and drew the calibration curve. 1 ml methanol extract of the sample (100µg/ml) was also mixed with the reagents and after 2 h the absorbance was measured. The total content of phenolic compounds in the extract of gallic acid equivalents (GAE) was calculated by the following formula: T= C.V/M, where, T= total content of phenolic compound, milligram per gram plant extract in GAE; C= concentration of gallic acid established from the calibration curve, milligram per milliliter; V= volume of extract, milliliter; M= weight of methanolic plant extract, gram.

Antioxidant Activity

The antioxidant activity was analyzed by estimating free radical scavenging activity following DPPH method of Kumar *et al.*[20] with modifications. 0.1mM solution of DPPH (1, 1-diphenyl-2-picrylhydrazyl), in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentration (10-100 µg/ml) and the absorbance was measured at 517 nm. Ascorbic acid at various concentrations (10-100 µg/ml) was used as standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation. DPPH scavenged (%) = Abs (control) - Abs (test)/ Abs (control) x 100, where Abs (control) is the absorbance of the control reaction and Abs (test) is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the leaf extracts was expressed as IC_{50} which is defined as the concentration (in µg/ml) of extract that scavenges the DPPH radical by 50%.

Statistical Analysis

The experimental results were expressed as mean ± standard deviation (SD) of triplicate measurements and the results were processed using microsoft excel and origin 2011.

Results and discussion

Five WLV collected from natural habitats and local markets in Sikkim were: (1) *Amaranthus viridis* Linn., family- Amaranthaceae; English name- pig weed; Nepali name- *Lattey sag/Lunde sag*; (2) *Chenopodium album* Linn., family- Chenopodiaceae; English name - Lamb's quarters/pig weed/goose foot; Nepali name - *bethu sag*; (3) *Diplazium esculentum* (Retz.) Sw., Family - Dryopteridaceae/Athyraceae; English name - fern; Nepali name - *ningro*; (4) *Nasturtium officinale* R.Br., family - Cruciferae; English name - watercress; Nepali name - *simrayo*; Lepcha name - *shamrock*; and (5) *Urtica dioica* Linn. Family - Urticaceae; English name - stinging nettle; vernacular name - *sisnoo* (Nepali), *sarong* (Lepcha). The collected samples of WLVs were analyzed for proximate composition (Table 1). The moisture content was > 90% in the leaves of *D. esculentum* and *N. officinale* and 86.7%, 84.8% and 84.5% in *A. viridis*, *C. album* and *U. dioica*, respectively (Table 1). These values were within the range estimated for some wild edible plants elsewhere[21]. Ash content varied from 12.2-24.9% (Table 1), which were in agreement with the results of Ajayi *et al*.[22] who reported an ash content of some leafy vegetables that ranged from 0.6-34%. Crude fibre in WLVs was 4.6-13.2% (Table 1). These values were within the range of 8.50-20.90% reported from some Nigerian vegetables[23]. Fat content was 3.4-9.6% in WLVs (Table 1). According to Handique[24] young leaves of *D. esculentum* contain low fat and a moderate amount of fibre. Protein content in WLVs was 19.8-33.8% (Table 1). The leaves and twigs of *U. dioica* have high content of protein[10]. Carbohydrate content varied from 31.7-64.6% (Table 1). Gopalan *et al*[25] has reported 7% to 32% protein and 20.0-66.8% carbohydrate in common leafy vegetables of India. The energy expressed in terms of calorific value (food value) varied from 343.0-376.7 kcal/100 g DM (Table 1). Potassium was the most abundant element in the samples (Table 1). This result agreed with the results reported for leafy vegetables[26]. Copper content estimated for *D. esculentum*, *N. officnale* and *U. dioica* was similar to those estimated for some leafy vegetables consumed in Kano of Nigeria by Mohammed *et al*.[27]. Iron content of WLVs was estimated within the range of 5.4-11.2 mg/100 g (Table 1). These values were found to be within the range estimated for some WLV of North East India[28]. Consumption of WLV as a source of micronutrients in many tropical areas is significant in small children's diet to ensure normal growth and intellectual development[29]. Sodium and potassium are important intracellular and extracellular cations, respectively[30]. The differences in the mineral content of the vegetable plant might be due to soil compositions and the rate of uptake of minerals by individual plant[31]. Vitamin C content in WLVs was recorded within the range of 3-44 mg/100 g (Table 1). Similar observations have been recorded by Gupta *et al*.[32] who reported 3-295 mg/100 g in thirteen locally available vegetables. The inhibition percentage values varied within the range of 53.2-

Table 1—Proximate, mineral composition and Vitamin C content of some WLVs of Sikkim

Parameters	Wild leafy vegetables				
	Amaranthus viridis	Chenopodium album	Diplazium esculentum	Nasturtium officinale	Urtica dioica
Moisture %	86.7 ± 0.5	84.8 ± 0.2	92.4 ± 0.1	90.6 ± 0.3	84.5 ± 0.1
Ash (% DM)	12.2 ± 0.9	19.5 ± 0.05	16.2 ± 0.7	24.9 ± 0.9	18.9 ± 0.3
Protein (% DM)	19.8 ± 0.6	23.0 ± 0.4	31.2 ± 1.0	33.8 ± 1.6	28.5 ± 1.3
Fat (% DM)	3.4 ± 0.5	4.2 ± 0.4	8.3 ± 1.3	9.6 ± 1.7	5.2 ± 0.4
Carbohydrate (% DM)	64.6 ± 1.7	53.3 ± 0.8	44.3 ± 1.5	31.7 ± 0.8	47.4 ± 1.3
Crude fibre (% DM)	8.2 ± 1.8	12.9 ± 0.1	4.6 ± 0.75	9.9 ± 0.7	13.2 ± 1.2
Food value (K cal/100g DM)	368.2 ± 1.3	343.0 ± 2.4	376.7 ± 1.9	348.4 ± 2.4	353.4 ± 3.0
Mineral (mg/100 g):					
Sodium	11.2 ± 3.5	7.4 ± 1.6	9.5 ± 2.2	68.8 ± 4.4	10.3 ± 2.8
Potassium	382.0 ± 15.4	848.32 ± 22.2	914.4 ± 23.5	465.2 ± 13.2	917.2 ± 26.3
Calcium	24.7 ± 2.1	155.75 ± 3.6	192.7 ± 5.2	65.6 ± 3.6	113.2 ± 7.5
Iron	10.8 ± 2.2	5.4 ± 1.3	11.2 ± 1.8	7.0 ± 0.92	8.1 ± 1.8
Copper	1.11 ± 0.2	1.22 ± .1	0.32 ± 0.04	0.58 ± 0.1	0.67 ± 0.03
Zinc	9.73 ± 1.02	8.44 ± 0.9	2.73 ± 0.1	2.04 ± 0.03	2.32 ± 0.1
Magnesium	0.48 ± 0.1	0.31 ± 0.1	0.36 ± 0.02	0.41 ± 0.1	0.22 ± 0.04
Vitamin C (mg/100 g)	44± 0.17	03± 0.1	21± 0.1	13± 0.1	07± 0.1

DM, dry matter; Data represents the means (± SD) of 10 samples each.

Table 2—Estimation of DPPH free radical scavenging activities (Inhibition %) of methanolic extracts some wild leafy vegetables of Sikkim

Conc. of extracts (μg/ml)	Wild leafy vegetables (Inhibition, %)					Ascorbic acid standard
	A. viridis	C. album	D. esculentum	N. officinale	U. dioica	
10	38.39 ± 0.96	33.50 ± 0.15	31.35 ± 3.02	29.09 ± 0.17	27.24 ± 0.50	91.99
20	43.25 ± 2.5	42.89 ± 1.74	42.98 ± 1.84	35.11 ± 1.09	32.05 ± 0.45	93.48
40	52.24 ± 2.4	47.93 ± 2.0	46.31 ± 0.44	43.83 ± 2.76	36.55 ± 0.39	96.58
60	56.56 ± 1.84	54.54 ± 3.21	52.02 ± 0.85	47.66 ± 2.69	41.95 ± 0.83	96.62
80	57.59 ± 2.71	58.63 ± 1.47	56.47 ± 1.30	53.19 ± 0.15	47.43 ± 1.54	96.76
100	61.87 ± 2.42	64.47 ± 0.81	57.95 ± 3.67	58.40 ± 1.15	53.19 ± 2.14	97.03

DPPH, 1,1-diphenyl-2-picryl hydrazyl. Data represents, mean (±.S.D) of 3 samples each

64.5% in WLV (Table 2). The DPPH free radical scavenging activities (inhibition percentage) were found to increase with increasing concentration and were estimated to be quite less than the standard (97.03% at 100 μg/ml). Maximum activity was observed in *Chenopodium album* (64.47%) (Table 2). The IC_{50} values were recorded as 46 μg/ml (*C. album*), 38 μg/ml (*Amaranthus viridis*), 70 μg/ml (*Nasturtium officinale*), 52 μg/ml (*Diplazium esculentum*), and 87 μg/ml (*Urtica dioica*). *A. viridis* and *C. album* are higher in antioxidant activity in comparison to *N. officinale, D. officinale* and *U. dioica*. At 10 μg/ml of methanolic extracts of plant samples the highest inhibition percentage was determined in *A. viridis* (38.39%) and lowest was recorded in *U. dioica* (27.24%). The methanolic extracts of selected five WLVs exhibited IC_{50} values below 100 μg/ml, indicating very good potential as free radical scavengers. Similar result has been reported in some uncommon vegetables of Pakistan[33]. A wide variation in the amount of total phenolic content ranging from 18-176 mg/g in GAE was observed in WLVs (data not shown). Highest TPC was determined in *U. dioica* (176 mg/g) followed by *C. album* (98 mg/g), *A. viridis* (98 mg/g), *N. officinale* (68 mg/g) and *D. esculentum* (18 mg/g). Several factors such as environmental, climatic, or geographic factors as well as extraction techniques may significantly influence the quality and the quantity of phenolic components present in nettle[34]. Although some studies have demonstrated a correlation between phenolic content and antioxidant capacity[35] in general our results show no correlation between total phenolic content and antioxidant activity. No correlation between total phenolic content and antioxidant capacities was also reported in many medicinal plant extracts[36]. No correlation between total phenolic content and antioxidant capacity in samples analysed was possible owing to the presence of the following factors: the antioxidant capacity observed was not solely from the phenolic contents, but could possibly be due to the presence of some other photochemicals such as ascorbic acid, tocopherol and pigments as well as the synergistic effects among them, which also contribute to the total antioxidant capacity.

Conclusion

The present study has shown that the WLVs are nutritionally rich in terms of calorific value, fibre, protein, mineral, low fat and vitamin C which altogether indicate the potentiality of the WLVs as good source of non-conventional vegetables. Due to their demonstrated nutritional qualities WLVs may help to overcome nutritional deficiency in local diets. The results on antioxidant activities and total phenolic content reasonably support their ethnomedicinal values. Based on the synergetic inference of all these results we believe to draw that WLV of Sikkim may be introduced for further intensive research including biological evaluation of the nutrient contents and subsequently in the line of domestication of WLVs.

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