NUTRIENT COMPOSITION, PHENOLIC CONTENT AND FREE RADICAL SCAVENGING ACTIVITY OF SOME UNCOMMON VEGETABLES OF PAKISTAN

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ABSTRACT

Vegetables play a vital role in the prevention of human disease and in the improvement of general health as these contain vitamins, amino acids, fiber, antioxidants and minerals. In the present study, some less familiar vegetables of Pakistan namely chickpea (leaves), chungah (shoots), drumstick tree (inflorescences), radish (fruit pods), mountain ebony (flower buds), mustard (leaves), purslane (leaves) and white goosefoot (leaves and shoots) were evaluated for proximate composition, mineral content, phenolic content and free radical scavenging activity. The protein, fat, fiber, carbohydrate and ash contents of the selected vegetables were in the range of 2.9 to 6.6%, 0.2 to 2.5%, 2.4 to 8.6%, 9.7 to 20.1% and 1.0 to 2.3%, respectively. The concentration of vitamin C ranged between 32.6 to 120.1 mg/100 g. The phosphorus, calcium, iron, zinc, manganese, magnesium and copper were 190 to 3400, 103 to 987, 19 to 93, 12 to 47, 9 to 121, 299 to 1635 and non detectable level to 42 mg/kg, respectively. The amount of total phenolic content varied from 55.3 to 221.0 mg/g in the dry methanolic extracts of the studied plants. The EC_{50} values were below 1400 µg/ml, indicating that all the studied vegetables have good scavenging effect on DPPH radical.

Keywords: Vegetables, nutrients, phenolic content, antioxidant activity.

INTRODUCTION

Plants are a rich source of healthy and important nutrients, as well as disease fighting constituents. Consumption of fruits and vegetables in diet protects the human body from chronic illnesses (Brown *et al.*, 1999; Christen *et al.*, 2008; Gosslau and Chen, 2004; He *et al.*, 2007; Heber, 2004; Hung *et al.*, 2004; Kavanaugh *et al.*, 2007; Lee *et al.*, 2008; Williams, 1995, Wintergerst *et al.*, 2006). The latest dietary guidelines suggest five to thirteen servings of fruits and vegetables a day, depending on caloric intake (USDA, 2005). Consumers are being increasingly encouraged to eat more fruits and vegetables as a contribution to a balanced diet and because a number of phytonutrients have been identified that may prevent diseases such as heart disease and cancer (Glew *et al.*, 1997; Szeto *et al.*, 2002; WCRF, AICR, 2007).

Phytonutrients have been getting greater consideration, as more and more research uncovers how powerful these nutrients are for health. This has mostly been attributed to the antioxidant and anti-proliferative effects of various phytochemicals present in high amounts in vegetables and fruits (Chu *et al.*, 2002; Proteggente *et al.*, 2002; Sun *et al.*, 2002). The vitamins A, C and E have already received considerable attention in this regard (Doering *et al.*, 1996). However, the majority of the antioxidant activities of plants are from phenolic acids and flavonoids (Bravo, 1998) etc. Numerous studies on health benefits linked with these compounds have been demonstrated (Badami and Channabasavaraj, 2007; Higdon and Frei, 2003; Willcox *et al.*, 2004). The prevention of cancer and cardiovascular diseases has been strongly related with the intake of fresh fruits and vegetables rich in natural antioxidants. This suggests that a higher intake of such compounds will lower the risk of mortality from these diseases (Willcox *et al.*, 2004).

Vegetables are among the important food crops as they supply ample amounts of flavonoids, phenolics, folic acid, ascorbic acid, carotene, riboflavin, and minerals like iron, calcium, zinc and phosphorous. A benefit of vegetable-rich diets has been partially attributed to the intake of variety of these phytochemicals. However, no single fruit or vegetables provide all of the nutrients, required for optimum health. The key lies in the consumption of the variety of different vegetables and fruits. In nature, there are many underutilized greens of promising nutritive value, which can well nourish the ever increasing human population. In search for sources of novel antioxidants and other important nutrients, a large number of plants have been extensively studied (Dasgupta and De, 2007; Indrayan *et al.*, 2005; Iqbal *et al.*, 2006; Islam *et al.*, 2004; Joyeux *et al.*, 1995; Odhav *et al.*, 2007; Xin *et al.* 2004) during last few years. Pakistan's diverse climatic condition offers an excellent opportunity of growing vegetables, fruits and condiments in all seasons throughout the year (GOP, MINFAL, 2006; Hanif *et al.*, 2006). In Pakistan, more than 63 varieties of vegetables distributed in 44 genera, are grown on large scale and consumed as summer and winter vegetables comprising mainly potatoes, gourds, tomatoes, cucumbers, lady

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fingers, turnips, cabbages, brinjal, cauliflowers etc. (Athar and Bokhari, 2006). These vegetables are popular for their freshness, taste and nutritive value.

Beside these, there are some other vegetables, which are comparatively less familiar and had been used mostly in rural areas by elders. However the younger's preference for these vegetables is consistently decreasing due to the lack of their awareness, limited availability and absence of popularization and utilization technologies. The knowledge of the ignored indigenous vegetables' consumption needs urgent scientific analysis and documentation. In the present study, some less familiar vegetables consumed largely by the rural populace of Pakistan were analysed for mineral contents, proximate composition, total phenolic content and free radical scavenging activity.

MATERIALS AND METHODS

Plant samples

Different edible parts of eight uncommon vegetables namely *Cicer arietinum* Linn. (chickpea), *Caralluma tuberculata* N.E. Brown. (chungah), *Moringa oleifera* Lam. (drumstick tree), *Raphanus sativus* Linn. (radish), *Bauhinia variegata* Linn. (mountain ebony), *Brassica campestris* Linn. (mustard), *Portulaca oleracea* Linn. (purslane) and *Chenopodium album* Linn. (white goosefoot) were purchased from the local market in Peshawar (table 1) and identified by Mr. Shahid Farooq, plant taxonomist, PCSIR Laboratories Peshawar. The plant samples were washed under running tap water. After that, the samples were spread on filter paper, cut into small pieces and dried at room temperature for 1–2 h. Proximate, mineral and vitamin C analysis were carried on fresh weight basis.

For free radical scavenging activity and total phenolic content, the leaves and young shoots of plants were dried in an air oven at 40±5°C for 8-10 h. The unripe pods of radish and shoots of chungah were dried in an air oven at 50±5°C. The dried plants samples were ground separately to fine powder in a Cyclotec mill of 60 mesh size. The powdered samples were kept in airtight containers at 4°C for further analysis.

Proximate and chemical analysis

The plant samples were analysed in triplicate for moisture, crude protein, fiber and ash on a fresh weight basis, according to AOAC (1990). The moisture was determined in a drying oven at 105 °C until constant weight. Analysis of crude fat was carried out using petroleum ether (bp. 40–60 °c) in a Soxtec system HT (Tecator). Determination of crude protein (% N x 6.25) was performed by the micro-Kjeldahl method. Ash contents were estimated by heating the samples at 550 °C and crude fiber by digestion with acid and alkali using Fibertec system (Tecator). Carbohydrates (%) contents were estimated by using a difference method (Odhav *et al.*, 2007), by subtracting the sum of the percent of moisture, fat, protein and ash from 100. The energy values of vegetables were calculated in kilojoules by multiplying the percentages of crude protein, crude lipid and carbohydrates by the factors 16.7, 37.7 and 16.7, respectively (Yadang *et al.*, 2009). The analysis for ascorbic acid was conducted by titration method using 2,6-dichlorophenol-indophenol.

Mineral analysis

The fresh samples of plants were evaluated by the method of wet digestion using a combination of nitric and perchloric acid for calcium, phosphorus, iron, zinc, manganese, copper, and magnesium. Each analysis was done in triplicate. About 1 g of each sample was digested in triplicate with a 10 ml of diacid mixture (HNO_3:$HClO_4$, 5:1, v/v) in a fume cupboard, heating initially at 80 °C and temperature was gradually increased to 250 °C. After complete digestion, each sample was heated to near dryness and volume was brought up to 50 ml with double distilled deionized water and filtered through Whatman No. 42 filter paper. Analysis was done using atomic absorption spectrophotometer and flame photometer.

Table 1: Vegetables used in this study

Scientific name	Family	Local name	English name	Parts used
Cicer arietinum Linn.	Fabaceae	Channa	Chickpea	Leaves and young shoots
Caralluma tuberculata N.E. Brown.	Asclepeadaceae	Chungah	Not available	Shoots
Moringa oleifera Lam.	Moringaceae	Suhanjna	Drumstick tree	Partially opened inflorescences
Raphanus sativus Linn.	Brassicaceae	Moli	Radish	Unripe fruit pods
Bauhinia variegata Linn.	Fabaceae	Kachnar	Mountain ebony	Unopened flower buds
Brassica campestris Linn.	Brassicaceae	Sarson	Mustard	Leaves
Portulaca oleracea Linn.	Portulacaceae	Kulfa	Purslane	Leaves
Chenopodium album Linn.	Chenopodiaceae	Bathu	White goosefoot	Leaves and young shoots

Preparation of extracts

The powdered samples of the vegetables were subjected to extraction (100 g each) by maceration in methanol (500 ml) at room temperature in dark for 10 days with occasional shaking. The macerates were filtered, and the filtrates were dried at low temperature (45°C) under vacuum in a rotary evaporator. The extracts were kept in dark at 4°C for further analysis.

Total phenolic content estimation

The total phenolic contents of plant samples were determined with Folin-Ciocalteau reagent (Khattak *et al.*, 2008). Appropriate dilutions of the extracts were prepared. In a test tube, 200 µl of the methanol extract was added to 4 ml of 2% aqueous sodium carbonate solution and mixed thoroughly. Then 200 µl of 50% Folin-Ciocalteau reagent was added. The mixture was allowed to stand for 1 hour with intermittent shaking and absorbance of the green-blue complex was measured at 750 nm in a spectrophotometer against blank. The spectrophotometric work was carried out using the Ultraspec 3000 UV/Visible spectrophotometer (Pharmacia). The total phenolic contents were calculated on the basis of the calibration curve of gallic acid. The results were expressed as milligram of gallic acid equivalents per gram (mg/g) of the dry extract.

Determination of DPPH radical scavenging activity

The antioxidant activity of the samples were assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Khattak *et al.*, 2008). It is free radical with a purple colour and has a maximum absorption at 517 nm. The free radial scavenging activity is based on the discoloration of the compounds when reduced by a free radical scavenger. About 100 µl of the sample at various concentrations was added to 2 ml of DPPH in methanol solution (60 µM) in a test tube and shaken vigorously. After incubation at 37 °C for 35 minutes in the dark, the absorbance of each solution was determined at 517 nm. The corresponding blank (control) reading was also taken. The activity was expressed as percentage scavenging of the DPPH by the plant extracts and calculated as:

% DPPH radical scavenging activity

$$= \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of sample}} \times 100$$

The results obtained from the radical scavenging experiments were expressed as EC_{50} values. EC_{50} value is the extract concentration at which 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals were reduced by 50% and calculated from the linear regression analysis.

STATISTICAL ANALYSIS

All determinations were obtained from triplicate measurements and results are expressed as means ± standard deviations. The data were analysed using one-way ANOVA for mean differences between the plant samples. The Statistical Package for Social Sciences (SPSS, version 14.0 for windows) was used to analyse the data. Statistical significance was declared at $p < 0.05$.

RESULTS

Nutrient composition

Eight less familiar Pakistani vegetables were investigated for their proximate composition and results are presented in table 2. Among all vegetables, the lowest value for moisture content was observed in white goosefoot (*Chenopodium album*) 71.3 %, whereas the highest was in mustard leaves (*Brassica campestris*) 85.6 %. Ash content in the selected vegetables varied from 1.0 to 2.3 %. The highest value for fiber content was found in radish unripe pods (*Raphanus sativus*) 8.6 % and lowest in purslane leaves (*Portulaca oleracea*) 2.4 %. White goosefoot leaves, chickpea leaves, drumstick tree inflorescences, mountain ebony flower buds, radish unripe pods and chungah shoots showed 6.2, 5.8, 3.2, 3.2, 2.5 and 2.5% fiber contents, respectively. The highest protein content were observed in chickpea leaves 6.6 %, followed by purslane 4.3 %, white goosefoot 3.9%, radish pods 3.5 %, drumstick tree 3.1 % and chungah shoots 2.9%. Mountain

Table 2: Proximate composition of selected uncommon vegetables

Plants	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrates (%)	Energy (kJ/100 g)
Chickpea	75.3 ± 1.3a	2.0 ± 0.1a	6.6 ± 0.3a	1.3 ± 0.1a	14.8 ± 1.5a	310.2 ± 21.5a
Chungah	83.4 ± 2.5b	1.0 ± 0.2b	2.9 ± 0.3b	0.2 ± 0.0b	12.5± 2.1a	224.1 ± 33.5ab
Drumstick tree	78.4 ± 1.4c	2.3 ± 0.2c	3.1 ± 0.2b	0.3 ± 0.0c	15.8 ± 1.1a	273.5 ± 17.7a
Radish	75.7 ± 1.9ac	1.8 ± 0.1d	3.5 ± 0.3b	0.2 ± 0.0b	18.8 ± 1.9ab	236.9 ± 27.5ab
Mountain ebony	78.1 ± 2.5ac	2.1 ± 0.3acd	2.9 ± 0.2b	0.5 ± 0.1d	16.4 ± 2.0a	287.0 ± 32.1a
Mustard	85.6 ± 2.0b	1.5 ± 0.2e	2.9 ± 0.3b	0.4 ± 0.1d	9.7 ± 1.6ac	181.4 ± 29.6b
Purslane	80.0 ± 2.9bc	1.8 ± 0.3ade	4.3 ± 0.6c	1.4 ± 0.2a	12.5 ± 2.2a	294.4 ± 35.5a
White goosefoot	71.3 ± 2.3d	2.2 ± 0.1c	3.9 ± 0.2bc	2.5 ± 0.1e	20.1 ± 2.2ab	390.3 ± 45.5c

The values presented in this table are on fresh weight basis. Values are mean ± standard deviations of three determinations. Means with different letters within the same column are significantly different ($p < 0.05$).

ebony and mustard both showed 2.9 % of protein content on fresh weight basis. The concentration of crude fat contents ranged from 0.2 to 2.5%. Highest value was recorded for white goosefoot leaves at 2.5 %. The amount of carbohydrates, in the selected vegetables varied between 9.7 to 20.1%. The vitamin C contents (fig. 1) of the vegetables ranged between 32.6 mg/100 g to 120.1 mg/100 g.

Mineral composition

The edible parts of plants were wet digested and analyzed for various minerals by using atomic absorption spectrophotometer (AAS) and flame photometer. The calcium contents in the plant samples were significantly varied ($p < 0.05$). The lowest value for calcium content was observed in partially opened inflorescences of drumstick tree (*Moringa oleifera*) 103 mg/kg, whereas the highest was in white goosefoot 987 mg/kg (table 3). The level of phosphorus contents in the selected vegetables varied from 190 to 3400 mg/kg. The chickpea pod showed the highest phosphorus content. Among all vegetables, the highest value for iron content was found in chickpea leaves (*Cicer arietinum*) 84 mg/kg and the lowest in chungah (*Caralluma tuberculata*) shoots 19 mg/kg. Drumstick tree, mustard, mountain ebony, purslane, white goosefoot and radish showed 87, 57, 53, 47, 47, and 43 mg/kg iron contents, respectively. The results of present analysis revealed that the vegetable samples contained zinc, manganese and magnesium in the range of 12 to 47, 9 to 121, and 299 to 1635 mg/kg, respectively. The highest zinc content were observed in purslane 47 mg/kg, followed by chungah 44 mg/kg, mountain ebony 37 mg/kg, chickpea 35 mg/kg, mustard 33 mg/kg, radish 31 mg/kg, white goosefoot 13 mg/kg, and drumstick tree (*Moringa oleifera*) 12 mg/kg. Manganese contents were highest in mustard leaves at 121 mg/kg and lowest in white goosefoot at 9 mg/kg. Highest level of magnesium content was recorded for drumstick tree partially opened inflorescences at 1635 mg/kg, followed by chickpea leaves 1400 mg/kg, white goosefoot leaves 1121 mg/kg, purslane leaves 870 mg/kg, mustard leaves 590 mg/kg, radish pods 567 mg/kg, mountain ebony flower buds 545 mg/kg, and chungah shoots 299 mg/kg. The highest amount of copper (42 mg/kg) was estimated in drumstick tree and the lowest in mustard leaves (11 mg/kg). No copper content were detected in mountain ebony unopened flower buds and white goosefoot leaves.

Phenolic content

The phenolic contents of the selected vegetable samples were determined using the Folin–Ciocalteau's phenol reagent (fig. 2). The results of current analysis revealed that leaves, pods and flower buds of plants contained a considerably high amount of phenolic ingredients and most of these were significantly different from each other ($p < 0.05$). Total phenolic contents ranged between 55.3 mg/g to 221.0 mg/g in the dry methanolic extracts of the studied vegetables.

DPPH scavenging activity

The free radical scavenging activity in the plant extracts was estimated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Results of DPPH reduction by methanolic extracts of plants are expressed as EC50 values and shown in fig. 3. A low EC50 value is the sign of strong antioxidant activity. The EC50 value of DPPH scavenging activity was varied from 41.7 to 1338.3 µg/ml and significantly different for all the extracts ($p < 0.05$).

DISCUSSION

Human beings need a variety of complex amalgam of organic compounds as supplementary caloric requirements to meet the need for routine biological activities. Plant materials constitute a big portion of the diet and therefore their proximate composition and nutritive value is especially important. Carbohydrates, fats and proteins are the major components of the diet. They provide energy (calories) and are important for growth, repair and metabolism. The nutritive value of the studied vegetables ranged between 181.4 (mustard leaves) to 390.3 (white goosefoot) kJ/100 g on fresh weight basis.

Table 3: Mineral contents (mg/kg) of selected un-common vegetables

Plants	Ca	P	Fe	Zn	Mn	Cu	Mg
Chickpea	430±23.2a	3400±93.3a	84 ± 3.0a	35 ± 1.1a	85 ± 3.8a	15 ± 0.4a	1400±80.3a
Chungah	233±12.6b	190±8.0b	19 ± 0.8b	44 ± 0.8b	86 ± 1.6a	35 ± 0.8b	299±33.6b
Drumstick tree	103± 4.6c	1860±53.3c	87 ± 4.0a	12 ± 0.5c	33 ± 0.9b	42 ± 2.2c	1635±85.6c
Radish	322±15.2d	934±49.2d	43 ± 1.5c	31 ± 1.3d	39 ± 1.8c	12 ± 0.7d	567± 48.2d
Mountain ebony	560±40.9e	540±25.5e	53 ± 1.6d	37 ± 2.2e	18 ± 0.6d	nd	545± 50.0d
Mustard	172±27.3f	1895±67.8c	57 ± 2.6d	33± 3.6de	121 ± 5.7e	11 ± 0.0e	590± 29.9d
Purslane	663±13.8g	774±65.6f	47 ± 1.1e	47 ± 2.0f	86 ± 3.7a	23 ± 0.8f	870± 36.5e
White goosefoot	987±71.8h	463±53.0e	47 ± 3.6ce	13 ± 1.2c	9 ± 0.2f	nd	1121± 76.4f

nd = not detected

The values presented in this table were on fresh weight basis. Values are mean ± standard deviations of three determinations.

Means with different letters within the same column are significantly different ($p < 0.05$).

To get maximum benefits regarding health, more and variety of vegetables should be included in food.

Vitamin C is very important for health (Duffy *et al.*, 1999; Fain, 2004; Grey, 1993; Iqbal *et al.*, 2004), as it form collagen in bones, cartilages, and muscles, and also helps in the absorption of iron. Dietary sources of vitamin C include a large number of vegetables and fruits. Among the studied vegetables, the highest amount of vitamin C (120.1 mg/100g) was estimated in drumstick tree followed by chickpea leaves 105.3 mg/100g, radish unripe pods 66.5 mg/100 g, mustard leaves 51.4 mg/100 g, purslane leaves 50.6 mg/100 g, mountain ebony flower buds 44.0 mg/100 g and white goosefoot leaves 43.7 mg/100 g (fig. 1). The lowest vitamin C content was recorded for chungah shoots 32.6 mg/100 g.

Minerals are essential to life as all living organisms use these to activate enzymes, hormones and other organic molecules that participate in the growth, various functions and maintenance of life processes (WHO, FAO, 2004). They cannot be synthesized and must be regularly provided. The present study showed that there are sufficient amounts of valuable minerals especially Fe, Zn, Ca and Mn in the studied plants. So these plants can be included in diet to have balanced nutrition.

Vegetable's phenolic antioxidants are effective in preventing the oxidative damage that may be the cause of arteriosclerosis, brain disorders, cancers and immune system deterioration (Ames, 1983; Steinberg, 1991). In the present study, the chickpea leaves showed the highest phenolic content at 221.0 mg /g, followed by mustard leaves 189.3 mg/g, radish unripe fruit pods 136.7 mg/g, mountain ebony flower buds 122 mg/g, purslane leaves 111.7 mg/g, white goosefoot leaves 91.0 mg/g, chungah shoots 69.0 mg/g, and drumstick tree inflorescences 55.3 mg/g. The phenolic content of mountain ebony and purslane were statistically same ($p > 0.05$). Increased dietary ingestion of natural phenolic compounds may reduce coronary heart diseases and have therefore a full range of perspective uses in healthcare. Earlier the phenolic components have shown an affinity to quench reactive oxygen species by flavonoids of *Gingko biloba* (Mahady, 2002), anthocyanins of fruits (Einbond *et al.*, 2004), tea catechins (Higdon and Frei, 2003) and flaxseed lignans (Westcott and Muir, 2003).

Free radicals are highly reactive chemical substances, which can lead to accelerated aging, cellular injuries, cancers, cardiovascular diseases and inflammations etc. Whole foods contain complex mixtures of antioxidants and are therefore responsible for many health benefits, and their advantage over single antioxidant is due to synergistic effects. The use of DPPH offers a simple and quick way to assess antioxidant activity. DPPH is stable at room temperature and produces a violet colour solution in ethanol. In the presence of antioxidant molecule it is reduced, giving rise to uncoloured ethanol solution. The methanolic extracts of three plants namely chickpea, mountain ebony and mustard exhibited EC_{50} values below 100 μg/ml, indicating a very good potential as free radical scavengers. Drumstick tree, white goosefoot, chungah and purslane leaves showed EC_{50} 376.0, 454.7, 695.7 and

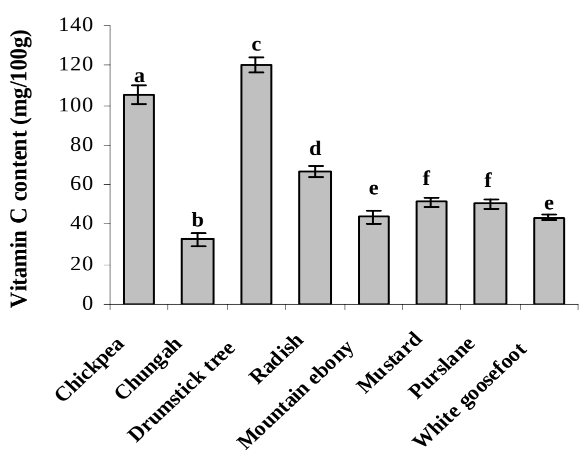

Fig. 1: Vitamin C contents of selected un-common vegetables on fresh weight basis. Values are mean ± standard deviations of three determinations. The vertical bars represent the standard deviations for each data point. Values with different superscript letters are significantly different ($p < 0.05$).

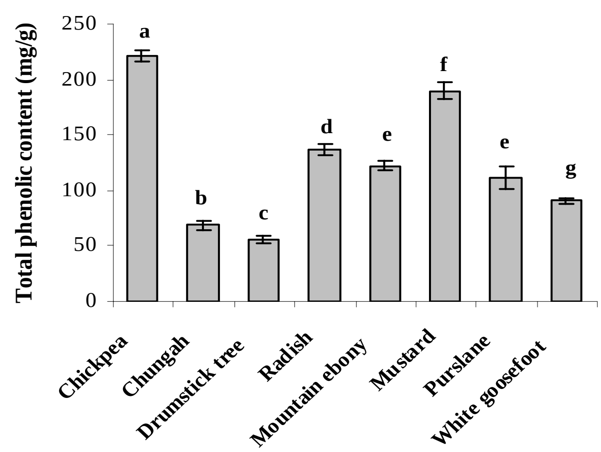

Fig. 2: Total phenolic content of the methanolic extracts (dry weight) in selected un-common vegetables. Values are mean ± standard deviations of three determinations. The vertical bars represent the standard deviations for each data point. Values with different superscript letters are significantly different ($p < 0.05$).

950.3 µg/ml, respectively. All the studied plants showed low EC_{50} values indicating strong free radical scavenging activity. Dietary polyphenolic compounds have a significant capacity to scavenge free radicals (Noguchi and Niki, 2000).

In general we have found no correlation between antioxidant activity and total phenolic content in the methanolic extracts of selected plants, as determined by squared regression co-efficient (R^2). Some plants showed high phenol contents but comparatively low DPPH activity.

Regardless of these advantages, unfortunately the majority of traditional vegetable plants are usually uncultivated and underutilized. It is critical to create awareness regarding diet related health benefits of these neglected precious crops. Further, with reference to food security there is a need to find out every potential source of safe and health-promoting nutrients.

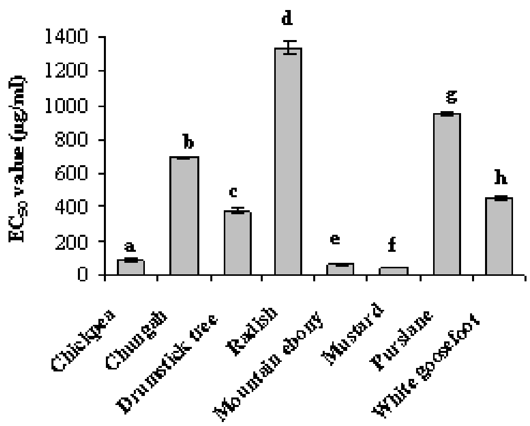

Fig. 3: DPPH scavenging activity expressed as EC_{50} value of the methanolic extracts of selected un-common vegetables. Values are mean ± standard deviations of three determinations. The vertical bars represent the standard deviations for each data point. Values with different superscript letters are significantly different ($p < 0.05$).

CONCLUSION

Findings of the study indicate that all the studied plants are excellent sources of micronutrients and free radical scavenging activity. The consumption of these traditional vegetables may have many beneficial health attributes. These data may also be helpful in allowing better food choices and improvement in nutritional and health status.

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