

# Antioxidant activity and nutritional assessment of under-utilized medicinal plants

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**Abstract:** The present study investigates the nutritive and anti-nutritive composition of six species (*Allium porrum*, *Amaranthus spinosis*, *Apium graveolens*, *Caralluma edulis*, *Chenopodium album*, *Urtica dioica*). The studied species contained considerable amount of crude protein (4.53-11.41%), crude fat (1.25-3.74%), vitamin C (7.85-28.09mg 100<sup>-1</sup>g) and  $\beta$ -Carotene (18.29-169.33mg 100<sup>-1</sup>g). The mineral profile of *Chenopodium album* is considered as potential source of Zn (14.51 $\pm$ 1.14mg 100<sup>-1</sup>g), Mn (67.71 $\pm$ 0.85mg 100<sup>-1</sup>g), Se (8.45 $\pm$ 0.49mg 100<sup>-1</sup>g) and Fe (182.08 $\pm$ 1.52mg 100<sup>-1</sup>g) and *Caralluma edulis* as potential source of Fe (7.28 $\pm$ 0.03mg 100<sup>-1</sup>g). Ca and P content ranged 63-306mg 100<sup>-1</sup>g and 12-392mg 100<sup>-1</sup>g in all studied plants with exceptionally high level was found in *Chenopodium album* (1084mg 100<sup>-1</sup>g) and (3924mg 100<sup>-1</sup>g), respectively. Similarly, total phenol, tannin and phytic acid were found in the range of 105-354, 5-90 and 10-85mg 100<sup>-1</sup>g, respectively. Total oxalates and soluble oxalates contents were below 200mg 100<sup>-1</sup>g in five examined plants with the exception of *Chenopodium album* (413mg 100<sup>-1</sup>g). Antioxidant activity of *Caralluma edulis* was found maximum while the rest of the examined plants had moderate levels.

**Keywords:** Nutritive value, wild plants, mineral composition, phytic acid, antioxidant activity.

## INTRODUCTION

The smooth access of foods is a solitary challenge for the inhabitants of Khyber Pakhtunkhwa province of Pakistan due to rapid population growth coupled with poverty and unabated natural and manmade crises. This situation was observed in many parts of the province including Peshawar, Swat, Bannu, Charsadda, Abbottabad, Bajaur and Khyber Agency suburbs. Limited livelihood opportunities due to the natural and man made disaster are the major constraints of food insecurity (Sher *et al.*, 2004). It is generally observed that most male community engaged in unskilled labor, while women are more likely to be self-employed in petty trade of agriculture especially in collection and trade of wild vegetables and medicinal plants. Food availability and household food security in mountain areas are closely related to agriculture, which is severely affected by the losses in both agriculture and livestock due to the current Afghan crisis. Moreover, the traditional ways of farming and livestock rearing have further reduced the income opportunity of the local people. A recent study confirms that all the above mentioned constraints strongly affect the purchasing power of local people (Sher and Hussain, 2009). Food is still the single most important commodity consumer's basket of goods and services, accounting for 80 percent of all expenditures in the area. Majority of these are "street foods", foods purchased and often consumed away from the household. In terms of current caloric intake, roughly 40 percent of households in the area could be classified as food insecure (Khalil, 2007). When current status is

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combined with a measure of vulnerability (proportion of total household budget devoted to food), 30 percent of households are classified as food-insecure, and an additional 70 percent are vulnerable (Sher and Hussain, 2009).

Various studies have stated that medicinal plants produce a huge amount of secondary metabolites having antioxidant activity and with antimicrobial effects on pathogens (Bakht *et al.*, 2011 a, b, c, d, 2012; 2013 a, b; 2014 a,b,c; 2015; Parveen and Bakht, 2015; Nasir *et al.*, 2015; Chaun *et al.*, 2015; Ullah *et al.*, 2015; Bilal and Bakht, 2016; Zakir *et al.*, 2015; Amjed *et al.*, 2016; Wajid *et al.*, 2016; Anwar *et al.*, 2016). *Allium porrum* (Gundana), *Amaranthus spinosis* (Chlwae), *Apium graveolens* (Ajmod), *Caralluma edulis* (Pamankay), *Chenopodium album* (Sarmay) and *Urtica dioica* (Seezonkay) are edible wild plants for many people of the study area. They are the key component in the regular diet during natural and manmade catastrophes. These species contain many bioactive nutrients in addition to conventionally identified nutrients such as protein, carbohydrates, vitamins and specific minerals, which might be responsible for the therapeutic action of the plants (Bray and Betteger, 1990, Cakmak and Marschner, 1988). In addition, element play a very important role in the formation of the active chemical constituents present in wild plants and are therefore responsible for their medicinal and nutritional properties (Rajurkar and Damame, 1998; Yang, 2009; Block, 2010;). They have high pharmaceutical value and are used as diuretic, astringent, carminative, appetizer, laxative and blood

purifier. They have strong potential to protect human body from hypercholesterolemia, cancers and cardiovascular disease (Alvarez, 2004; Kruger *et al.*, 1998). Recognizing the importance of such plants the present work was conducted to analyze the proximate composition, selected minerals, total tannin contents, B-carotene and vitamin C of selected plants. The data will be used to estimate the nutritional as well as pharmacological intake of population of the study area and in future clinical trials.

## **MATERIALS AND METHODS**

### ***Study area***

The six plants species for the present study were collected from various parts of Khyber Pakhtunkhwa Province of Pakistan including Peshawar, Swat, Bannu, Charsadda, Abbottabad, Bajaur and Khyber Agency suburbs. The study area ranged from dry rocky areas in the south of KP to forests and green plains in the north. The climate of the subject area included extreme of both intensely hot summers to freezing cold winters.

### ***Sample collection and sample preparation***

The individual plant sample was collected from the seven mentioned study area (its natural habitat) at early to middle mature stage. They were identified and voucher specimens of *Allium porrum* L. (PES-2353), *Amaranthus spinosus* L. (PES-1057), *Apium graveolens* L. (PES-2032), *Caralluma edulis* (Edgew.) Benth. & Hook.f (PES-1214), *Chenopodium album* L. (PES-2119) and *Urtica dioica* L. (PES-5412) were deposited in Herbarium of Pakistan Forest Institute, Peshawar. Representative samples from edible portion of each species were prepared by randomly picked compositing 3-5kg of each plant and mixed thoroughly. The selected plants were immediately washed off dust, dirt and other impurities using tap water until no foreign material remained, drained completely at room temperature for less than 2h. The plant material was then dried under shade for 5 to 7 days due to minimum loss of bioactive compounds. The air dried plant material was then ground by electrical grinder (Yigan, Model WF130) and the powdered samples were sieved from 80 mm mesh sieve. The powdered samples were packed in plastic bags sealed and stored at 4°C for subsequent biochemical analysis. They were analyzed for their proximate composition, selected mineral contents, vitamin C, β-carotene, oxalates, polyphenols, total tannin content and antioxidant activity.

### ***Proximate composition***

Moisture, ash, Crude fat, crude fiber and crude protein were determined by standard method of AOAC (2000). Nitrogen Free Extract (NFE) was calculated by subtracting the sum of the percentages of moisture, crude fiber, crude protein, crude fat and Ash from 100 (McDonald *et al.*, 1973; Iqtidar and Saleemullah, 2004).

$NFE = 100 - \% (\text{Moisture} + \text{crude fat} + \text{crude protein} + \text{fiber} + \text{Ash})$

### ***Energy calculation***

The caloric value of each plant sample was calculated from the energy yielding components i.e. Fats, Protein, Carbohydrate. The crude fat was multiplied by 9.1 and the sum of protein and carbohydrates was multiplied by 4.3. The sum of resulting figs. (Fat, protein and carbohydrate) will be Kilo calories (K cal) per 100gm sample (AOAC 2000; Iqtidar and Saleemullah, 2004).

### ***Vitamin-C estimation***

The vitamin C was determined by the dye reduction method. For this purpose the ascorbic acid content of the sample was titrated with 2, 6 dichlorophenol indophenol as described in AOAC (2000).

### ***Determination of beta carotene***

β-Carotene was isolated by column chromatography and estimated Spectrophotometrically using the modified method of Noelle and Grivetti (2002).

### ***Mineral analysis***

The dried powdered samples were first digested with nitric acid and perchloric acid and then the aliquots were used for the determination of phosphorus by spectrophotometer and mineral nutrients (Se, Zn, Cu, Mn, Fe, Ca, Mg) by atomic absorption spectrophotometer (AOAC, 2000).

### ***Anti-nutritional factors***

Total oxalate contents were determined by extracting with hydrochloric acid and soluble oxalate with water followed by precipitation with calcium oxalate from deproteinized extract and subsequent titration with potassium permanganate (Gupta *et al.*, 2005). Total phenol contents in the methanolic extracts were assessed using a modified version of the Folin-Ciocalteu assay. Gallic acid was used as a standard (Li *et al.*, 2008). Tannins level in the methanolic extracts was assessed using the vanillin-HCl method of Waterman and Mole (1994). Similarly, pytic acid was determined using the method of Vaintraub and Lapteva (1988).

### ***Anti-oxidant activity***

Anti-oxidant activity was determined by using the 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay as described by Choi *et al.* (2002). DPPH (1mL, 0.3mM) solution in ethanol was added to 2.5mL (100 mg mL<sup>-1</sup>) of methanol extract and mixture was allowed to react for 30 min. at room temperature and absorbance was recorded using UV visible spectrophotometer at 518 nm. HPLC grade Rutin hydrate (95%, 1mM) was used as standard, while absolute ethanol was used as negative control. The experiment was repeated in triplicate, using fresh plant sample each time and the average absorbance values were converted to percentage anti-oxidant activity.

**Table 1:** Analysis of different dietary ingredient of selected wild vegetables of Khyber Pakhtunkhwa province

Subject Plants	Moisture	Ash	Crude Protein	Crude Fiber	Crude Fat	NFE	Energy Content (Cal 100 <sup>-1</sup> g)	β-Carotene	Vitamin C
	Percentage							mg 100 <sup>-1</sup> g	
<i>Allium porrum</i>	50.58 ±3.53	3.97 ±1.47	11.41 ±2.55	4.83 ±1.39	3.74 ±1.13	25.47 ±5.47	197.67	82.17 ±4.47	28.09 ±3.94
<i>Amaranthus spinosis</i>	77.80 ±4.09	2.85 ±0.15	4.53 ±1.13	7.39 ±3.53	3.34 ±1.23	4.09 ±2.45	67.46	169.33 ±16.26	17.60 ±3.55
<i>Apium graveolens</i>	72.98 ±5.64	1.84 ±0.13	5.60 ±2.12	14.72 ±2.82	2.60 ±1.16	2.26 ±1.23	67.44	46.67 ±276.06	26.39 ±4.26
<i>Caralluma edulis</i>	62.44 ±3.72	2.77 ±0.22	10.66 ±3.74	17.47 ±4.68	2.64 ±1.66	4.02 ±2.51	87.13	48.33 ±55.75	23.57 ±3.82
<i>Chenopodium album</i>	75.33 ±7.45	2.37 ±1.45	9.08 ±3.49	6.59 ±2.89	1.25 ±1.13	5.38 ±3.18	73.55	118.29 ±0.32	18.29 ±2.32
<i>Urtica dioica</i>	67.19 ±4.77	3.07 ±0.13	6.68 ±3.13	15.38 ±3.10	2.58 ±1.42	5.10 ±3.10	74.12	74.33 ±7.77	7.85 ±1.98

**Table 2:** Phytochemical/Anti-nutritive compounds and anti-oxidant activity of examined plants.

Subject Plants	Total oxalates	Soluble oxalates	Phytic acid	Tannins	Total phenols	Antioxidant activity
	mg 100 <sup>-1</sup> g					Percentage
<i>Allium porrum</i>	38.67±2.03	13.33±1.45	12.01±1.04	60.00±1.52	132.33±32.86	5.14±0.36
<i>Amaranthus spinosis</i>	180.87±4.85	99.86±3.27	85.27±3.48	70.83±2.08	105±4.57	5.9±1.68
<i>Apium graveolens</i>	56.66±3.17	23.00±1.15	22.05±1.78	5.67±0.58	177.00±1.52	32.54±1.42
<i>Caralluma edulis</i>	165.66±3.48	120.33±1.45	25.56±1.48	90.67±0.58	110.66±4.97	59.83±2.66
<i>Chenopodium album</i>	413.33±3.17	131.66±2.18	63.47±2.13	2.11±3.48	354.66±4.70	52.95±1.15
<i>Urtica dioica</i>	26.33±2.72	18.66±2.96	10.43±1.33	66.67±5.77	142.66±1.85	26.13±2.02

**Table 3:** Elemental analysis in selected wild vegetables collected from Khyber Pakhtunkhwa province (mg 100<sup>-1</sup>g).

Elements	<i>Allium porrum</i>	<i>Amaranthus spinosis</i>	<i>Apium graveolens</i>	<i>Caralluma edulis</i>	<i>Chenopodium album</i>	<i>Urtica dioica</i>
Zn	21.76±0.91	0.47±0.04	0.50±0.02	0.78±0.03	14.51±1.14	0.41±0.04
Cu	105.50±2.49	0.02±0.01	0.12±0.05	0.17±0.03	0.21±0.01	0.07±0.05
Mn	60.96±1.48	0.81±0.01	0.42±0.02	0.64±0.05	67.71±4.85	0.46±0.04
Se	3.32±0.21	1.96±0.01	1.23±0.01	1.49±0.01	8.45±2.49	1.25±0.01
Fe	49.26±0.51	6.14±0.18	2.36±0.15	7.28±0.03	182.08±5.52	1.82±0.70
Ca	63.66±2.72	175.29±0.28	175.46±0.22	306.64±0.25	1084±10.96	153.00±0.04
Mg	1852.67±8.90	7.84±0.09	8.57±0.06	10.84±0.34	311.00±2.30	6.48±0.06
P	3236.33±5.92	15.47±0.15	12.30±0.10	17.50±0.20	3924.33±8.81	14.50±0.10

± represent Standard Error (SE)

Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

$$\% \text{ Radical scavenging activity} = (\text{control OD} - \text{sample OD} / \text{control OD}) \times 100.$$

IC<sub>50</sub> value is the concentration of the sample required to scavenge 50% DPPH free radical.

### STATISTICAL ANALYSIS

The experiment was repeated in triplicate, using fresh plant sample each time and the average absorbance values were converted to percentage anti-oxidant activity.

MSTAT computer software was used for the analysis of the data. Standard Error was calculated for each sample (Steel *et al.*, 1997).

### RESULTS

The data revealed that the moisture content of the *Amaranthus spinosis* was maximum (77.80%) followed by that of *Chenopodium album* (75.33%), whereas minimum level of moisture was found in *Allium porrum* (50.58%). *Allium porrum* was venerable source of crude Protein (11.41%) and crude fat (3.74%). Similarly, *Apium graveolens* was moderate source of crude fiber (14.72%)

and *Allium porrum*, a good source of ash (3.97%). The maximum amount of calculated energy was found in *Allium porrum* (197.67 Cal 100<sup>-1</sup>g), followed by *Caralluma edulis* (87.13 Cal 100<sup>-1</sup>g). Similarly, mean value of the data (table-1) showed that highest amount of NFE was found in *Allium porrum* (25.47%) followed by *Chenopodium album* (5.38%) and *Urtica dioica* (5.10%) whereas the lowest was observed in *Apium graveolens* (2.26%). Our data also revealed that all the examined species contained Vitamin C and  $\beta$ -Carotene in moderate amount. It was observed that maximum level of beta-carotene was found in *Amaranthus spinosis* (169.33 mg 100<sup>-1</sup>g) followed by *Chenopodium album*. The analysis of the data (table 2) revealed that total phenol, total tannin and phytic acid are found in the range of 105-354, 2-90 and 10-85 mg 100<sup>-1</sup>g, respectively. All examined plants are excellent sources of total phenol including *Chenopodium album* (354.66 $\pm$ 4.70 mg 100<sup>-1</sup>g) and *Apium graveolens* (177 mg 100<sup>-1</sup>g) whereas *Caralluma edulis* (90.67 $\pm$ 0.58mg 100<sup>-1</sup>g) and *Amaranthus spinosis* (9070.83 $\pm$ 2.08mg 100<sup>-1</sup>g) are good sources of total tannin contents. (118.29 $\pm$ 0.32mg 100<sup>-1</sup>g) and *Allium porrum* (82.17 mg 100<sup>-1</sup>g), whereas minimum level in *Apium graveolens* (46.6mg 100<sup>-1</sup>g). The maximum value of phytic acid was found in *Amaranthus spinosis* (85.27 $\pm$ 3.48 mg 100<sup>-1</sup>g). The investigated plants had total oxalates and soluble oxalates in reasonable level. Results of the present study (table 2) showed that total oxalates were found in the range of 26-380mg 100<sup>-1</sup> g and soluble oxalates in the range of mg 13-131mg 100<sup>-1</sup>g. The results (table 2) revealed that the maximum value of percent antioxidant activity was observed in *Caralluma edulis* (59.83%), whereas minimum level in *Amaranthus spinosis* (5.9%).

## DISCUSSION

Wild plants have been recognized for the common dweller of Khyber Pakhtunkhwa province Pakistan as the cheapest and promising source of food, especially during the time of flood/famine and other natural and unabated conflicts. Each sample in the present study was investigated for their nutritive and anti-nutritive constituents to evaluate their nutritional potential adequacy (table 1-3). Moisture content of the *Amaranthus spinosis* was more compared with other plant species under study. *Allium porrum* was venerable source of crude protein and crude fat. Similarly, *Apium graveolens* was moderate source of crude fiber and *Allium porrum*, a good source of ash. The maximum amount of calculated energy was found in *Allium porrum*. Similar results are also reported by Aletor *et al.* (2002), Hussain *et al.* (1984), Kaushalaya *et al.* (1989) and Sundrial and sundriyal (2000) who depict approximate range of ash (0.75-3.7%) crude protein (0.05-30.53%), crude fat (0.56-4.5%) and crude fiber (1.5-10.5%) for various wild edible plants like *Amaranthus*, *Caralluma*, *Chenopodium* etc

species. Highest amount of NFE was found in *Allium porrum* whereas the lowest was measured in *Apium graveolens*. These NFE results agree with the earlier work of Khalil and Durrani (1989) and Aberoumand and Deokule (2008) who recorded 15 to 40% NFE on fresh weight basis in conventional and nonconventional vegetables including *Carulluma* and *Apium* species. Our data also revealed that all the examined species contain Vitamin C and  $\beta$ -Carotene in moderate amount as compared to data reported by various researchers (Agte *et al.*, 2000, Anchana *et al.*, 2005). It was observed that maximum level of beta-carotene was found in *Amaranthus spinosis* whereas minimum in *Apium graveolens*. A plethora of research studies oscillates that a number of vitamins (vitamin-C, vitamin-B,  $\beta$ -Carotene, tocopherol), minerals (Fe, Se and Zn) and various phytochemical constituents have prominent role in the prevention of cardiovascular, cancer, cataracts, aging diseases and act as quencher of radicals/antioxidants (Brown *et al.*, 1999; Claude 2003; Penny *et al.*, 2002; Rajua *et al.*, 2007).

Total phenol, total tannin content, phytic acid, total oxalates and soluble oxalates are phytochemical compounds, which have nutritional, therapeutic and anti-nutritional significance. The analysis of the data revealed that total phenol, total tannin and phytic acid are found in the range of 105-354, 2-90 and 10-85 mg 100<sup>-1</sup>g, respectively. All examined plants were excellent sources of total phenol including *Chenopodium album* and *Apium graveolens* whereas *Caralluma edulis* and *Amaranthus spinosis* were good sources of total tannin contents. The maximum value of phytic acid was found in *Amaranthus spinosis*. Likewise, it was found that the investigated plants had total oxalates and soluble oxalates in reasonable level when compared to other conventional and non-conventional plants as reported (Shingade *et al.*, 1995; Gupta *et al.*, 2005). Results of the present study indicated that total oxalates were found in the range of 26-380mg 100<sup>-1</sup> g and soluble oxalates in the range of mg 13-131mg 100<sup>-1</sup>g. Nevertheless, these anti-nutrients were also found in other conventional dietary plants in varying amount (Wallace *et al.*, 1998), however, available literature stresses the nutritional and medicinal significance of these compounds as well whose presence greatly impair the digestion of various nutrients and responsible for several health complications (Obob and Akindahunsi, 2004; Gupta *et al.*, 2005; Gidamis *et al.*, 2003). Nevertheless, these phytochemicals are considered valuable sources of antioxidants. In the present study, the subject food plants are not the sole source of anti-oxidant but might be the effective contributor of anti-oxidant. Maximum value of percent antioxidant activity was observed in *Caralluma edulis*, whereas minimum level in *Amaranthus spinosis*. Similar results are also reported by Romojaro *et al.* (2013) while studying 13 species of wild edible plants consumed in the Mediterranean diet.

Similarly, the all the six studied plants were investigated for selected minerals including Zn, Cu, Mn, Se, Fe, Ca, Mg and P. It was observed that these plants are promising source of minerals. Iron and zinc are present in appreciable amount ranging 1.82-182.08 and 0.41-21.76mg 100<sup>-1</sup>g, respectively. According to WHO Iron and zinc are among the essential elements for humans and their daily requirements for adult are 15 and 18 mg respectively. Manganese content varied from 0.42 (*Apium graveolens*) to 67.71mg 100<sup>-1</sup>g in *Chenopodium album*. Magnesium and Calcium was found in minimum concentration in *Urtica dioica*, highest quantity in *Allium porrum* and *Chenopodium album*. Similarly, selenium and manganese were found maximum in *Chenopodium album*. Similar results are also reported by Akpanyung et al., (1995), Barminas et al. (1999) and Kumari et al. (2004).

## CONCLUSION

In many parts of the studied area access to balance and nutraceutical rich foods are not possible. Local people mostly rely on wild dietary for their routine food requirements. This preliminary research work showed that these six plants are good source of protein, fiber, carbohydrates, minerals, vitamins and bioactive compounds. Hence, the present reclaim emphasizes the importance of wild vegetables along with the conventional vegetables. This study was a part of extensive project on the nutritive and antioxidant potential of wild and conventional plants and their possible application in Agricultural system.

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