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## Nutritional, phenolic content and antioxidant activities of vegetables introduced under protected cultivation at high altitude areas of Tawang, Arunachal Pradesh, India

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### Abstract

A field experiment was conducted for nutritional profiling of vegetables at Defence Research Laboratory, Research and Development Centre (DRL R&D Centre), Tawang during winter season cultivated under protected conditions. The vegetables ash content (%) varied from 6.05 – 13.52; moisture content (%): 6.68 – 18.39; crude protein (%): 9.29 – 44.39, crude fat (%): 0.65 – 1.148, crude fibre (%): 10.18 – 19.64, and carbohydrate (%): 33.43 – 76.11 respectively. The energy level of vegetables ranged from 291.78 – 351.59 Kcal with a good source of essential minerals. The total phenolic content of vegetables varied from 12.83 to 27.34 mg GAE/g extract. The highest antioxidant activity was observed in red cabbage with IC<sub>50</sub> value of 68.33±0.31 µg/mL followed by carrot, broccoli and radish. Thus, vegetables are nutritionally rich in terms of fibre, protein, ash, mineral, antioxidants and phenolic content, which indicate the potential of these vegetables as a good source of nutrition.

**Keywords:** Nutrition, antioxidant, vegetable, minerals

### 1. Introduction

It is a fact that health is wealth. The health of a person depends on the qualities and quantities of food stuff one consumes. Vegetables play a vital role in human diet and consumption of these vegetables provide us essential nutrients component such as carbohydrates, protein, dietary fiber, mineral element, vitamins, antioxidant and other beneficial phytochemicals (Prakash and Pal, 1991) <sup>[1]</sup>, (Khattak and Rahman, 2017) <sup>[2]</sup>. The World Health Organization recommended that adult should consume at least 400 g of fruits and vegetables per day. It has been observed that high daily intake of fruits and vegetables promote good health and reduced risk of disease (Boeing *et al.* 2012) <sup>[3]</sup>. Vegetables are fresh and edible portion of the herbaceous plants are available almost throughout the year in a wide variety. They can be eaten raw, cooked and pickled, which have excellent nutritional properties and cheaper source of energy. They play an important role to the rural poor and tribal communities in the form of food and nutrient supplement. These vegetables will continue to remain the basic source of energy for the developing countries (Akwaowo *et al.* 2000) <sup>[4]</sup>.

Now a day's food demands have been increasing day by day with the exponential human population growth resulting in marginal land resource availability for growing food crops especially vegetables. According to the Food and Agricultural Organization (FAO), there are about 840 million undernourished people in 1998–2000, of whom 799 million are in developing countries, 30 million in the countries in transition and 11 million in the industrialized countries. To apprehend the situation, interests have been centralized on the exploitation, quantification and utilization of food plants, especially the vegetables (Hussain *et al.* 2010) <sup>[5]</sup>.

Tawang is a medium sized town in western part of Arunachal Pradesh of domain of India, situated at 33°35'13N 71°26'29E with an altitude of 9, 000 feet. Tawang region, a semiarid region, having fewer land resources to the locals to satisfy their food demands through agriculture. Along with the seasonal crop, people are dependent on vegetables for their nourishments.

In the present study four vegetable species viz. broccoli (*Brassica oleracea* var. italica, cv. Fiesta), red cabbage (*Brassica oleracea* var. capitata, cv. Scarlet o' hara), radish (*Raphanus sativus* var. Longipinnatus, cv. local), European carrot (*Daucus carota* subsp. Sativus, cv. Karoda Improved), cultivated under protected conditions in customised green house of DRL R&D Centre, Tawang, were collected to study their nutritional properties. The selected species were assayed for their proximate (moisture, ash, crude fiber, crude proteins, fats, and carbohydrates), phenolic content, antioxidant activities and mineral element (Na, K, Ca, Fe, Mg, Mn, Cu, Ni, and Co) contents.

## 2. Materials and Methods

### 2.1 Materials

Commercial cultivars of broccoli (cv. Fiesta), red cabbage (cv. Scarlet o' hara), radish (cv. local) and European carrot (cv. Karoda Improved) were planted under customised greenhouse during winter season (September 2019 to December 2019) at DRL (DRDO) R&D Centre, Tawang, Arunachal Pradesh which is located at an altitude of 9300 feet. The vegetable samples were collected randomly for the proposed analysis. Samples were dried and grounded according to standard protocol and laboratory grade chemicals were used for the estimation of parameters. The edible parts of collected samples were separated and washed with water. The samples were sliced in to small fragments and dried in hot air oven at 50°C for 24 h. The dried material obtained was grounded to a fine powder with a grinder and kept in air tight containers for further analysis.



(a) Red cabbage (cv. Scarlet o' hara)



(b) Broccoli (cv. Fiesta)



(c) European carrot (cv. Kuroda Improved)



(d) Radish (cv. local)

**Fig 1:** Cultivated vegetables in high altitude areas of Tawang, Arunachal Pradesh

### 2.2 Proximate analysis

The proximate composition (moisture, ash, crude fat, crude fiber, and crude protein) of the samples were determined using the standard methods of the Association of Official Analytical Chemist (AOAC, 2000) [6]. Ash was determined in silica crucibles by incineration in a muffle furnace (KK Scientific Supplier, Model No: MF03, Korea.) at 550°C for 5 hours. The nitrogen contents were determined by micro Kjeldahl method (Kjelplus 20 L Pellican Equipment, India) involving digestions, steam distillation and finally titration with standard 0.01 M HCl solution. The nitrogen content is converted to crude protein by multiplying a factor of 6.25. Carbohydrate content was estimated by difference method as described by (James, 1995) [7]. The total energy value in

kcal/100g was estimated using the formula described by (FAO, 2003) [8].

$$\text{Calorific value (kcal)} = (a * 4) + (b * 4) + (c * 9)$$

- amount of carbohydrate in g,
- amount of protein in g,
- amount of fat in g

### 2.3 Mineral content analysis

The calcium, sodium, potassium, magnesium, zinc, copper, iron, manganese, nickel, and cobalt were determined by wet digestion and analysed using atomic absorption spectrophotometer (Thermo Scientific, ICE 3000 Series,

Newington, USA). The concentration of minerals were calculated by comparison of absorption of samples against known concentration of working standards and results were converted to mg/100 g. (AOAC, 2000) <sup>[6]</sup>.

#### 2.4 Determination of total phenolic content of vegetables

The total phenolic content (TPC) was estimated by Folin-Ciocalteu reagent method (Singleton *et al.* 1999) <sup>[9]</sup> with minor modification. The extracted each sample (50 µl) was incubated with Folin Ciocalteu reagent (125 µl) for 5 min. 125 µl of 7.5% of Na<sub>2</sub>CO<sub>3</sub> was added to the mixture and volume was made to 1ml with distilled water. It was then incubated for 2 hours at room temperature. Absorbance was measured at 760 nm against distilled water as blank and result was designated as mg of gallic acid equivalents milligram per gram of extract. Analysis was carried out in triplicate.

#### 2.5 Determination of antioxidant activity of vegetables

Antioxidant activity was measured using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) technique as explained by Dasgupta *et al.* (2007) <sup>[10]</sup> with minor modification. 100 µl of standard ascorbic acid or prepared sample (1-100 µg/mL) was added to 1 ml of 0.01 mM methanolic DPPH solution and incubated for 35 min in dark at room temperature. Absorbance was measured at 517 nm in UV-Spectrophotometer (Specord-251). DPPH scavenging percentage is calculated as

$$\%DPPH \text{ scavenging} = \left( \frac{(\text{Control absorbance} - \text{Extract absorbance})}{\text{Control absorbance}} \right) * 100$$

#### 2.6 Statistical analysis

Experimental data obtained was analysed with SPSS Statistics 21.0 software. One-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) was used for significance test, considering  $p \leq 0.05$  statistically significant. Analysed data were represented as mean  $\pm$  SD obtained in three replicates ( $n = 3$ ) from different experiments.

### 3. Results and Discussion

#### 3.1 Proximate composition

The results obtained for proximate analysis of the samples shows different concentration/proportions of biochemical and other contents which are shown in table 1. The results indicated that the moisture content in four vegetable species ranged from 6.68% to 18.39%. Red cabbage was having the highest (18.39%), while radish having the lowest moisture content on dry weight basis (6.68%) (Table 1). High moisture content helps in maintaining the protoplasmic contents of the cells and also supports a greater activity of water-soluble enzymes and co-enzymes needed for metabolic activities of these vegetables. On the other hand, high moisture content makes vegetables susceptible to spoilage (Gbadamosi *et al.* 2011) <sup>[11]</sup>. Ash content in vegetables is an indication of the probable amount of mineral elements present in the vegetables. (Fagbohun *et al.* 2012) <sup>[12]</sup>. High ash content in a vegetable would indicate high mineral content, hence very nutritious. Ash content of vegetables varied from 6.05% for Carrot to 14.42% for radish (Table 1). Results indicated that the vegetable might

be considered as good source of minerals. Protein helps in the body building and repair of damage tissue, therefore it is considered as an important part of human diets (Onwordi *et al.* 2009) <sup>[13]</sup>. The crude protein content in the samples varied widely from 9.29% to 44.39% as shown in Table 1. As a result, all investigated vegetables excluding carrot (9.29%), could be recommended as source of protein (Prakash and Pal, 1991) <sup>[1]</sup> and (Boeing *et al.* 2012) <sup>[3]</sup>.

The crude fat contents ranged from 0.65% to 1.48% with a lowest content in red cabbage and the highest in broccoli. Fat is good source of energy and a medium for dissolving vitamin A, B, E and K and its deficiency may results in suboptimal growth; however consuming excessive amount may lead to fatty liver problem and susceptibility to respiratory disease (Ogunmoyela *et al.* 2013) <sup>[14]</sup>. Dietary fat is a major determinant of palatability of food. It has also been reported that vegetable fats and oil lower blood lipids, hence contribute to reduction in the occurrence of diseases associated with damage of coronary artery (Anita *et al.* 2006) <sup>[15]</sup>. Vegetable fibre is known to cleanse the digestive tract, remove potential carcinogens from the body, as well as keep blood sugar levels under control. In this study the highest crude fibre content was found in radish (19.64%) and lowest in red cabbage (10.18%). The carbohydrate contents varied from 33.43% for broccoli and 76.11% for carrot. Carbohydrate supply energy for nutrition and they are readily fermented by micro-organisms to yield carbon dioxide, alcohol, organic acids and other compounds (Emebu *et al.* 2011) <sup>[16]</sup>. Gross energy values of the vegetables under study were determined. The highest gross energy value of 351.59 kcal is observed for Carrot. The smallest value of 291.78 kcal was determined for red cabbage.

#### 3.2 Mineral nutrient contents

Sodium, potassium, calcium, and magnesium are considered as macro-nutrients, whereas iron, zinc, copper, manganese, cobalt and nickel are micro-nutrients in human diet, which are also known as trace elements. The results of micronutrients analysis of the vegetables species showed significant variation among different micronutrients (Table 2). Among the macronutrients, potassium was found to be in highest quantity followed by calcium, sodium, magnesium and in case of micronutrients iron was dominant with respect to manganese, nickel and copper. However, under this investigation the presence of cobalt was not detected in any one of the four vegetable species. In case of potassium, it was highest in radish ( $4132.22 \pm 8.88$  mg/100 g) while lowest in carrot ( $1657.00 \pm 18.37$  mg/100 g). Nair *et al.* (2013) <sup>[17]</sup> reported that presence of high quantity of potassium in our diet helps in lowering blood pressure. In radish excellent amount of calcium was recorded ( $1631.52 \pm 7.76$  mg/100 g) and the rest of vegetables varied from  $717.03 \pm 15.60$  mg/100 g for broccoli to  $957.60 \pm 13.05$  mg/100 g for red cabbage. Calcium helps in building and maintenance of bones and teeth strength. It plays a vital role in blood clotting, muscles contraction, and neurological function and also helps in enzymatic metabolic processes (Saha *et al.* 2015) <sup>[18]</sup>. The sodium contents in vegetable species varied from  $27.03 \pm 0.142$  mg/100 g for red cabbage to  $935.93 \pm 7.16$  mg/100 g for radish. Sodium helps in fluid distribution, blood pressure, and cellular work. The magnesium content in radish is highest among all the vegetable species, having value of (532.01 mg/100 g)

followed by red cabbage, which has value of 248.81 mg/100 g (Table 2). The iron content is highest in broccoli (11.98 ± 0.33 mg/100g) and lowest in red cabbage (5.37 ± 0.236 mg/100g). The recommended daily intake of iron for adult male is 8 mg and for adult female is 18 mg (Sajib *et al.* 2014) [19]. Manganese content of all the vegetables ranged from 1.76 ± 0.051 to 2.85 ± 0.026 mg/100 g. Manganese is an essential component of metalloenzymes and it is involved in various physiological processes by activating some enzyme, which is necessary for the metabolism of carbohydrate, cholesterol and amino acid. (Sajib *et al.* 2014) [19]. The copper content of vegetables under study ranged between 0.94 ± 0.07 mg/100 g for radish to 1.64 ± 0.05

mg/100 g for carrot. Copper is one of important nutritional trace element for the manufacture of enzyme found in human body and play a vital role in biological electron transport. Copper deficiency may cause increase in oxidative stress and induces numbers of biological process such as reduced energy production, abnormal glucose and cholesterol metabolism. It has been reported that copper complexes have an anticancer, antiulcer, and anti-diabetic properties (Saha *et al.* 2015) [18]. The level of nickel content in the sample varied from 1.07 ± 0.29 mg/100 g to 2.55 ± 0.293 mg/100 g while in red cabbage it was not detected. However, presence of cobalt was not detected in any of the vegetables.

**Table 1:** Proximate composition of vegetables under study at high altitude areas of Tawang

Parameters (%)	Broccoli	Radish	European carrot	Red cabbage
Moisture	12.54 ± 0.27 <sup>c</sup>	6.68 ± 0.31 <sup>a</sup>	7.44 ± 0.38 <sup>b</sup>	18.39 ± 0.54 <sup>d</sup>
Ash	8.16 ± 0.12 <sup>b</sup>	13.52 ± 0.02 <sup>d</sup>	6.05 ± 0.02 <sup>a</sup>	9.47 ± 0.043 <sup>c</sup>
Crude protein	44.39 ± 0.02 <sup>d</sup>	14.42 ± 0.10 <sup>b</sup>	9.29 ± 0.15 <sup>a</sup>	19.69 ± 0.202 <sup>c</sup>
Crude Fat	1.48 ± 0.02 <sup>d</sup>	0.8 ± 0.05 <sup>b</sup>	1.11 ± 0.02 <sup>c</sup>	0.65 ± 0.0504 <sup>a</sup>
Crude fibre	11.39 ± 0.06 <sup>b</sup>	19.64 ± 0.81 <sup>c</sup>	11.87 ± 0.81 <sup>b</sup>	10.18 ± 0.11 <sup>a</sup>
Carbohydrate	33.43 ± 0.13 <sup>a</sup>	64.58 ± 0.48 <sup>c</sup>	76.11 ± 0.27 <sup>d</sup>	51.53 ± 0.48 <sup>b</sup>
Calorific value (kcal)	324.61 ± 0.72 <sup>b</sup>	323.25 ± 1.12 <sup>b</sup>	351.59 ± 1.51 <sup>c</sup>	291.78 ± 2.54 <sup>a</sup>

Values are represented as mean ± standard deviation. Values followed by different superscript letter in a row are significantly different (p≤0.05).

**Table 2:** Mineral composition of vegetables

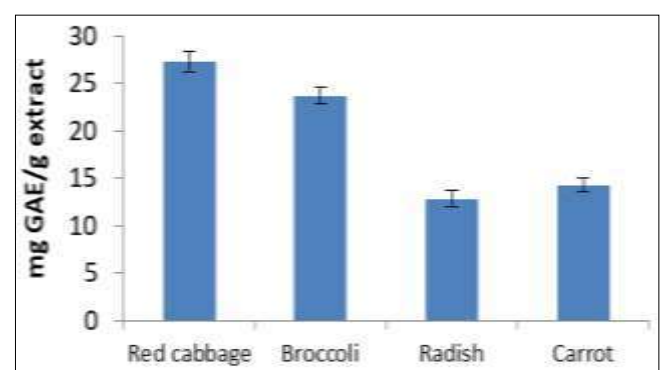
Macronutrients present in vegetables (mg/100g)				
Elements	Broccoli	Radish	European carrot	Red cabbage
Na	286.61 ± 8.95 <sup>b</sup>	935.93 ± 7.16 <sup>d</sup>	834.75 ± 3.83 <sup>c</sup>	27.03 ± 0.142 <sup>a</sup>
Ca	717.03 ± 15.60 <sup>a</sup>	1627.71 ± 8.56 <sup>d</sup>	908.33 ± 9.67 <sup>b</sup>	956.81 ± 9.32 <sup>c</sup>
K	2965.17 ± 26.11 <sup>b</sup>	4131.65 ± 6.35 <sup>d</sup>	1657.00 ± 18.37 <sup>a</sup>	3481.26 ± 2.12 <sup>c</sup>
Mg	242.22 ± 0.96 <sup>b</sup>	532.01 ± 3.68 <sup>d</sup>	188.83 ± 4.28 <sup>a</sup>	248.81 ± 3.032 <sup>c</sup>
Micronutrients present in vegetables (mg/100g)				
Elements	Broccoli	Radish	European carrot	Red cabbage
Fe	11.98 ± 0.33 <sup>c</sup>	6.65 ± 0.11 <sup>b</sup>	6.64 ± 0.11 <sup>b</sup>	5.37 ± 0.236 <sup>a</sup>
Mn	2.23 ± 0.058 <sup>c</sup>	1.76 ± 0.051 <sup>a</sup>	2.85 ± 0.026 <sup>d</sup>	2.01 ± 0.025 <sup>b</sup>
Cu	0.98 ± 0.045 <sup>a</sup>	0.94 ± 0.066 <sup>a</sup>	1.42 ± 0.046 <sup>b</sup>	1.64 ± 0.044 <sup>c</sup>
Ni	2.55 ± 0.293 <sup>c</sup>	1.07 ± 0.29 <sup>a</sup>	1.85 ± 0.23 <sup>b</sup>	ND
Co	ND	ND	ND	ND

Values are represented as mean ± standard deviation. Values followed by different superscript letter in a row are significantly different (p≤ 0.05); ND: Not Detected.

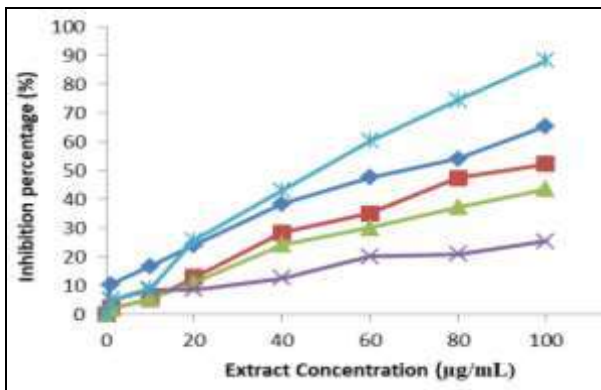
### 3.3 Total phenolic content and antioxidant activity of vegetables

The phenolic content and antioxidant activity of vegetables are depicted in Figure 2 and 3. The phenolic content of vegetables was calculated from the standard calibration curve using the regression equation ( $R^2 = 0.9986$ ,  $y = 0.0058x + 0.06$ ), expressed in gallic acid equivalent mg/g of extract. The total phenolic content of vegetables under study varied from 12.83 to 27.34 mg GAE/g extract (Figure 2). Among the four vegetable species red cabbage was found to have highest phenolic content (27.34 mg GAE/g extract) followed by broccoli (23.71 mg GAE/g extract), carrot (14.28 mg GAE/g extract) and radish (12.83 mg GAE/g extract) respectively. Results obtained in this study were similar to those reported by Stratil *et al.* (2006) [20]. In present investigation, the competence of vegetable extract to scavenge DPPH was determined on the basis of their concentration executing 50% inhibition ( $IC_{50}$ ). Among the four vegetable species red cabbage was found to have highest antioxidant activity with  $IC_{50}$  value of 68.33±0.31 µg/mL, followed by carrot ( $IC_{50} = 87.98 ± 0.28$  µg/mL),

broccoli ( $IC_{50} = 108.29 ± 0.34$  µg/mL) and radish ( $IC_{50} = 212.15 ± 0.51$  µg/mL) respectively (Figure 3). The radical scavenging activity of vegetable extract may be due to its phenolic compounds. Results obtained in the present study were in accordance to those reported by Dasgupta *et al.* (2007) [10].



**Fig 2:** Mean total phenolic content of vegetables extract



**Fig 3:** DPPH scavenging capacity of vegetable extract and ascorbic acid standard

#### 4. Conclusion

The present study indicated that the vegetables cultivated in high altitude are nutritionally rich in terms of fibre, protein, ash, mineral, having high antioxidant activity and phenolic content, which all together indicate the potential of the vegetable as a good source of nutrition. It was observed that nutrient composition in all vegetables were different, however, the calorific value is almost in equal level. So it can be concluded that the vegetables can be recommended for general cultivation under protected conditions in addition to traditional vegetables of Tawang and can be consumed as complement of each other. In the present study, it was observed that broccoli, red cabbage, carrot and local radish contain high amount of macro nutrient and significant amount of micro nutrient. Results indicated that broccoli, red cabbage, carrot and local radish are good in antioxidants and phenolic content. Hence, broccoli, red cabbage, carrot and radish can be cultivated successfully in high altitude areas of Tawang to diversify the dietary pattern of the dwellers.

#### 5. Acknowledgement

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#### 6. Conflict of interest

Authors declare that they have no conflict of interest.

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