Applied Biological Research 23(2): 209-214; (2021)
DOI: 10.5958/0974-4517.2021.00027.6 **SHORT COMMUNICTAION**

QUANTIFICATION OF ASCORBIC ACID, BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY IN SOME UNCONVENTIONAL LEAFY GREENS

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ABSTRACT

The present study was aimed to explore the antioxidant potential of some unconventional leafy greens, namely turnip, radish, cauliflower and carrot. These leafy greens, procured from a local vegetable market, were analyzed for ascorbic acid content using AOAC protocol. The standard chemical assay methods were used to ascertain the contents of bioactive compounds namely chlorophyll, total carotenoids, flavonoids and total phenols in leaves. Anti-oxidant activity was estimated based on DPPH assay. The study revealed that ascorbic acid content in fresh leaves ranged from 62.85 to 66.27 mg 100 g^{-1} with highest contents in turnip and lowest in cauliflower leaves. The chlorophyll (164.5 mg 100 g^{-1}) and total carotenoids contents (5.59 mg 100 g^{-1}) were significantly ($p \leq 0.05$) high in cauliflower leaves. Radish had significantly higher flavonoid (400.6 mg QE 100 g^{-1}) and total phenols (231.9 mg GAE 100 g^{-1}) as compared to the other leaves. The antioxidant activity in leaves ranged from 14.76 to 77.52% with highest value in carrot and lowest in cauliflower. The study revealed that fresh leaves of these vegetables as a good source of bioactive compounds may add diversity and anti-oxidants to the diets that may protect the body from oxidative stress and free-radical damage.

Keywords: Antioxidants, carrot, cauliflower, radish, turnip, unconventional leafy greens

INTRODUCTION

Worldwide a total of 1,097 vegetable species are cultivated and we are just familiar with <7% of these species (Meldrum *et al.*, 2018). The rest are underutilized, underexploited, unconventional, minor or rare vegetables, which are mainly consumed in tribal or indigenous communities. Our diets are less diverse and based on a few crops. In contrast, one-third of world food [equivalent to 1.3 billion tons] is either lost or wasted each year (FAO, 2019). In nature, there are many underexploited greens with good nutritive value, which can nourish the ever-increasing human population. Many of these are tolerant to adverse climatic conditions. India is blessed with diverse agroclimatic conditions with a vast resource of vegetable greens, many of which are still under-exploited (Kowsalya and Mohandas, 1999). Unconventional greens are inexpensive and high-quality nutritional sources for the low population segment, especially where malnutrition is widespread (Nnamani *et al.*, 2009).

Phenolics compounds are most abundant antioxidants in our diet (Vuolo *et al*., 2019). Structural characteristics of phenols, such as the presence of double bonds, number and position of the hydroxyl group, glycosylation, and the presence of substituents in the ring, determine their antioxidant power (Wang *et al*., 2018). Unconventional greens being a rich source of phytochemicals and phenolic compounds, contribute enormously to the antioxidant activity in diet (Uusiku *et al*., 2010). Consumption of such vegetable greens is confined to the people living in areas where they grow. The nutritive value of commonly consumed leafy vegetables has been studied extensively, but there is very little information on the nutritive value of unconventional leafy vegetables (Pattan and Devi, 2014). Many vegetable greens, for instance the leaves of carrot, cauliflower, chickpea, colocasia, radish, and turnip, are rich in micronutrients and antioxidants but are usually discarded (Joshi and Mathur, 2010), and thus add to the vegetative waste. These unconventional greens are readily available in vegetable market at no cost. These are not consumed by general masses; rather are used as cattle feed. Increasing the consumption of unconventional greens in our diet can be a food-based approach for treating the micronutrient deficiency. Intake of unconventional greens in daily diet can be used as one of the strategies to improve the nutritional status. The unconventional vegetable greens are gaining importance as a means to enhance per capita availability of food; hence, they can be explored to improve the nutritional scenario in the country.

In India, both malnutrition and poverty are widespread. There is a need to explore the feasibility of unconventional and underutilized vegetable greens to overcome malnutrition problems. Unconventional vegetable greens being a reservoir of essential vitamins, micronutrients, proteins and other phytonutrients have vital role in attaining the nutritional security. The nutritive potential of some less commonly consumed inexpensive vegetable greens has not yet adequately been studied (Joshi *et al.*, 2020). Therefore, the present study was aimed to assess the ascorbic acid, bioactive compounds, and antioxidant activity of unconventional leafy greens namely turnip, radish, cauliflower and carrot.

MATERIALS AND METHODS

Procurement of vegetable greens

About 1 kg fresh leaves of some vegetables, namely, turnip (*Brassica rapa*), radish (*Raphanus sativus*), cauliflower (*Brassica oleracea*), and carrot (*Daucus carota*), were procured from a local vegetable market in December, 2017.

Preparation of samples

The fresh green leaves of above selected vegetables were brought to the Nutrition laboratory of the Department of Food and Nutrition, PAU, Ludhiana (India). The diseased and rotten leaves and debris were discarded and the rest samples were washed with tap water to remove soil and other materials. The leafy vegetables were subject to chemical analysis.

Estimation of ascorbic acid

The leafy vegetable samples were separately analyzed for ascorbic acid by AOAC method (2000). One gram sample was ground along with 20 mL of 6% metaphosphoric acid in a pestle and mortar. The slurry was filtered, then 30 mL metaphosphoric acid added to the residue and filtered again. Three separating funnels (50 mL) were set and labeled as A, B, and C; A for sample, B for dye and C for standard. The filtrate (5 mL) was pipetted into separating funnel A, 0.1 mL standard ascorbic acid solution was put into funnel C. Then acetate buffer (5 mL) was added to all three funnels followed by the addition of 2 mL dye solution. Xylene (10 mL) was quickly added and the contents shaken for 5-10 sec. The contents were allowed to separate into two layers. The bottom layer was discarded. The xylene layer was transferred to a test tube and optical density read in a double beam UV/VIS spectrophotometer - 2375) at 500 nm using the formula:

$$\text{Ascorbic acid (mg)} = 0.1\,(b-a) / (b-c)$$

Where b = OD of blank; a = OD of sample; c = OD of standard

Chlorophyll and total carotenoids

The chlorophyll and total carotenoid contents were determined as per Thimmaiah (1999). For this, 1 g sample was homogenized with 10 mL of 80% acetone and centrifuged at 5000 rpm for 5 min to collect the filtrate. The residue was again extracted with 80% acetone until the residue became colourless. The final volume was made to 100 mL with 80% acetone, and its absorbance measured at 663, 645 and 480 nm on a double beam UV/VIS spectrophotometer. The chlorophyll content and total carotenoids were calculated using the following formula:

$$\text{mg total chlorophyll } (g^{-1} \text{ tissue}) = 20.2\ (A_{645}) + 8.02(A_{663}) \times V/1000 \times W$$

$$\text{mg total carotenoids } (g^{-1} \text{ tissue}) = A_{480} + 0.114\ A_{663} - 0.638\ A_{645} \times V/1000 \times W$$

Where A = absorbance at a specific wavelength; V= final volume of the extract in 80% acetone, and W= fresh weight of the tissue extracted.

The chlorophyll content was expressed in terms of mg 100 g^{-1} sample.

Determination of flavonoids

The aluminum chloride colorimetric method (Woisky and Salatino, 1998) was adopted to determine the flavonoids in the samples. The sample (1 g) was extracted with 25 mL of 95% ethanol under constant shaking at 200 rpm for 24 h. After filtration, the filtrate was adjusted to 25 mL with 80% ethanol. The extract (0.5 mL) was taken in a test tube and 0.5 mL 96% ethanol, 0.1 mL 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL distilled water were added. The mixture was incubated at room temperature for 30 min. The absorbance of mixture was read at 415 nm in a double beam UV/VIS spectrophotometer. For preparation of a calibration curve, 10 mg quercetin was dissolved in 80% ethanol and then diluted to 25, 50 and 100 µg mL^{-1}. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL 10% aluminum chloride, 0.1 mL 1 M potassium acetate and 2.8 mL distilled water. Then the absorbance was read at 415 nm in a double beam UV/VIS spectrophotometer.

Estimation of total phenols

The leaf samples were analyzed for total phenols using Folin-Ciocaltue reagent method (Singleton *et al*., 1999). For this, 1 g sample was extracted with 20 mL of 80% methanol by shaking for 3 h and the process repeated twice. The extract was centrifuged at 10000 rpm for 5 min and the supernatant filtered through Whatman No. 1 filter paper. The final volume was made upto 50 mL with 80% methanol and the methanolic extract stored at - 20°C till analysis. The methanolic extract (0.5 mL) was taken in a test tube, the volume made to 1 mL with 0.5 mL of 80% methanol and then 5 mL Folin-Ciocaltue reagent added and shaken thoroughly for 5 min. Then 4 mL saturated solution of sodium carbonate was added to the mixture before incubation in dark at room temperature for 2 h. The absorbance of mixture was read at 765 nm in a spectrophotometer against a blank. The concentration of total phenols was read from the standard curve, prepared by using gallic acid in the range of 20 to 100 µg mL.

Antioxidant activity

The free radical scavenging activity was determined by using the DPPH assay (0.1 mM) (Dehshahri *et al*., 2012). For this, 2 g sample was extracted with 20 ml methanol under a constant shaking for 2 h. The extraction process was repeated twice. The extract was then centrifuged at 10000 rpm for 15 min. Then to 100 µL extract, 2.9 mL DPPH solution was added and the mixture vortexed for 1 min before incubation in dark for 3 min. The discoloration of DPPH was measured against a blank at 517 nm in a double beam UV/VIS spectrophotometer. The DPPH scavenging activity was measured using the formula:

$$\text{Inhibition in DPPH radicals (\%)} = B - S/B \times 100$$

Where B = absorbance of blank; S = absorbance of the sample

Statistical analysis

All the experiments were conducted in triplicates. The mean and standard deviations for various parameters were computed as per the standard method of Gomez and Gomez (1984). The analysis of variance was employed to assess significant difference in the concentration of bioactive compounds using Microsoft Excel (2003) Statistical Analysis Tool Pack. Least significant difference (LSD) at 5% was calculated for comparison among the parameters.

RESULTS AND DISCUSSION

The ascorbic acid content in fresh leaves of turnip, radish, cauliflower and carrot ranged from 62.85 to 66.27 mg 100 g^{-1} with highest content in turnip leaves (Table 1). The ascorbic acid content (64.26 mg 100 g^{-1}) in radish leaves was found in line with the ascorbic acid content of 65.76 mg 100 g^{-1} reported by Longvah *et al.* (2017). The higher ascorbic acid content in cauliflower leaves observed in present study is almost similar (52.84 mg 100 g^{-1}) to Longvah *et al.* (2017). Alam *et al.* (2020) reported vitamin C content in the range of 22.08 to 59.01 mg 100 g^{-1} in unconventional leafy vegetables such as Holud Pata, Kachuripana, Orhor Pata, Shorna Lata, Amrul, Sada Koroi, and Telakucha.

Table 1: Ascorbic acid and bioactive compounds in unconventional leafy greens

Chemical constituents	Fresh leaves				$LSD_{0.05}$
	Turnip	Radish	Cauliflower	Carrot	
Ascorbic acid (mg 100 g^{-1})	66.27 ± 0.60	64.26 ± 0.35	62.85 ± 2.43	63.86 ± 1.81	ns
Chlorophyll (mg 100 g^{-1})	129.04^{a} ± 1.21	97.90^{b} ± 8.17	164.53^{c} ± 3.19	103.31^{b} ± 6.51	12.89
Total carotenoids	4.69^{a} ± 0.35	3.48^{b} ± 0.38	5.59^{c} ± 0.04	4.10^{a} ± 0.03	0.61
Flavonoids (mg QE 100 g^{-1})	337.12^{a} ±11.69	400.61^{b} ± 7.96	317.12^{a} ± 8.79	340.00^{ac} ± 8.34	21.86
Total phenols (mg GAE 100 g^{-1})	219.52^{a} ±13.93	231.90^{a} ± 1.80	170.95^{b} ± 8.93	175.71^{b} ± 2.58	19.77

Values are mean ± SD; Mean of nine replicates; NS: Non-significant; Superscripts with same alphabets imply non-significant difference at 5% level of significance. Superscripts with different alphabets imply a significant difference at 5% level of significance

The ascorbic acid content in the selected leaves showed no significant difference; however, a significant ($p \leq 0.05$) difference was observed in bioactive compounds such as chlorophyll, total carotenoids, flavonoids and total phenols and their antioxidant activity. The highest chlorophyll content was found in cauliflower leaves (164.53 mg 100 g^{-1}) and lowest in radish leaves (97.9 mg 100 g^{-1}). Cauliflower leaves also possessed significantly higher total carotenoid contents (5.59 mg 100 g^{-1}) as compared to the other vegetable leaves. The least total carotenoids were found in radish leaves (3.48 mg 100 g^{-1}). Our results are supported by Gocher *et al*. (2017) who reported 109 mg chlorophyll in 100 g cauliflower leaves. Ankita and Prasad (2015) reported 105.76 and 3.96 mg chlorophyll and β-carotene per 100 g fresh radish leaves, respectively. Agarwal and Varma (2014) also observed 3.4 mg chlorophyll g^{-1} fresh radish leaves. Ashraf *et al*. (2016) observed 0.71 mg chlorophyll and 0.46 mg total carotenoids in 100 g fresh radish leaves. Sam *et al*. (2020) reported high total carotenoid content in unconventional leafy vegetables of Bangladesh such as Parul, Helencha, and Roktrodone.

The radish leaves had significantly higher flavonoids (400.61 mg QE 100 g^{-1}) in comparison to the carrot leaves (340 mg QE 100 g^{-1}), turnip leaves (337.12 mg QE 100 g^{-1}) and cauliflower leaves (317.12 mg QE 100 g^{-1}) [Table 1]. Radish leaves possessed maximum total phenols (231.90 mg GAE 100 g^{-1}) while minimum was noticed in cauliflower leaves (170.95 mg GAE 100 g^{-1}). AbdEl-Fattah (2014) has reported 808.55 mg total flavonoids and 1196.8 mg total phenols 100 g^{-1} fresh turnip leaves, respectively. The value of 27.57 and 28.94 mg total polyphenols 100 g^{-1} fresh radish and cauliflower leaves, respectively, has earlier been observed by Longvah *et al.* (2017).

The evaluated leafy vegetables exhibited widely varied in their anti-oxidant activity (14.76 to 77.52%) [Fig. 1]) with highest activity noticed in carrot and lowest in cauliflower leaves. The antioxidant activity of turnip leaves was 44.34% which was much lesser than the value of 90.37% reported by AbdEl-Fattah (2014); however, a closer value of 45-50% DPPH radical inhibition has

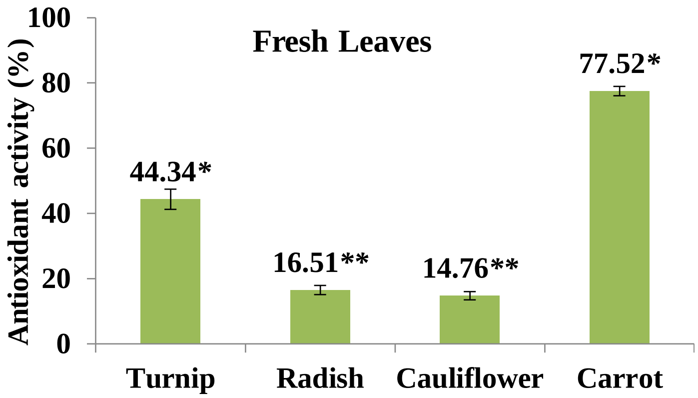

Fig. 1: Antioxidant activity of unconventional leafy greens; LSD at 5%: 4.57; *significant at 5% level of significance; **non-significant at 5% level of significance

been reported by Reddy *et al*. (2010). Shehata *et al*. (2014) have reported the antioxidant activity of 20.5, 19.2 and 31.5% in fresh radish, carrot and cauliflower leaves, respectively. The study revealed that unconventional vegetable greens possess good nutritive value and have tremendous potential to overcome deadly diseases. The fresh leaves of turnip, radish, cauliflower and carrot can be used as dietary source of anti-oxidants. Their inclusion in diets may add to the diversity in daily food intake, taste and flavour in diet. Incorporation of these leaves in daily diet may protect consumers from oxidative stress and free radical damage. There is need to conduct studies on animals to assess the impact of these leaves on oxidative stress markers. Anti-oxidant-rich food products can be developed by adding these leafy greens in various forms. The proper use of unconventional leafy greens in the form of vegetable leaf powder (Joshi *et al*., 2019) can be a reasonable effort towards achieving the nutritional security of the masses at a low cost.

Author's contribution: The conceptualization of research work and designing of experiments was done by 2nd author (KB), the execution of field/laboratory experiments and data collection by 1st author (NJ), the analysis of data and its interpretation by NJ & KB and the preparation of manuscript by NJ.

Declaration: The authors declare that there is no conflict of interest.

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