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COMPARATIVE ANALYSIS OF CARBOHYDRATE, PROTEIN, ASCORBIC ACID, PHENOLIC, FLAVONOID AND ANTIOXIDANT EVALUATION OF RED AND GREEN COLOR *AMARANTHUS* LEAFY VEGETABLE FROM INDIA

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ABSTRACT

The *Amaranthus* microgreen is an inexpensive and excellent sources of phytonutrients, with good flavor, and texture. Microgreens have short growth cycle and thus, can be grown without soil and without external inputs like fertilizers and pesticides. In the present study, the carbohydrate, protein, ascorbic acid, phenolic, flavonoid and Antioxidant were evaluated and compared in red and green color *Amaranthus* leafy vegetable. The protein levels were significantly higher in red (0.020±0.0024mg/ml) than green *Amaranthus* (0.016±0.008mg/ml) p(≤0.05). The ascorbic C, flavonoids, phenolics levels and antioxidant in green was statistically higher than red *Amaranthus* p (≤0.05).

Keywords: red *Amaranthus*, green *Amaranthus*, phenol, flavonoid, antioxidants

INTRODUCTION

The *Amaranthus* a plant with versatile uses such as ornamental plants, vegetables, and grains distributed worldwide including Africa, America, Asia, Australia, and Europe. *Amaranthus* is widely used in traditional medicines especially as antiviral, antimalarial, antidiabetic, antibacterial, antihelminthic and snake antidote. *Amaranthus* species are cheap vegetables containing good protein with lysine and methionine, dietary fibre vitamins carotenoids, and minerals such as calcium, magnesium, potassium, phosphorus, iron, zinc, copper, and manganese **[1]**.

Amaranthus are rich source of antioxidant pigments, such as betalain, β-xanthin, and β-cyanin compared to other leafy vegetables and an excellent source of other antioxidant pigments, such as anthocyanins, carotenoids, and chlorophylls. It also contains various natural antioxidant phytochemicals, such as vitamin C, phenolic acids, Twenty-five flavonoids and phenolic acids such as phydroxybenzoic acid, salicylic acid, protocatechuic acid, vanillic acid, gentisic acid, gallic acid, β-resorcylic acid, ellagic acid, syringic acid, chlorogenic acid, m-coumaric acid, trans-cinnamic acid, caffeic acid, ferulic acid, sinapic acid, p-coumaric acid, naringenin, rutin, isoquercetin, kaempferol, catechin, hyperoside, myricetin, apigenin, and quercetin **[2]**.

There are two colours in *Amaranthus*, one is red and another is green. Red *Amaranthus* has more pigments than green *Amaranthus*. Red colour *Amaranthus* are an excellent source of pigments, phytochemicals such as β-TPC, β-xanthin, TFC, cyanin, betalain, carotenoids, and vitamin compared to green colour genotype.

The *Amaranthus* microgreens can easily be grown in urban or peri-urban settings and even inside residential area where land is often a limiting factor due to their short growth cycle, without soil and without external inputs like fertilizers and pesticides. The carbohydrate, protein total phenolic content, flavonoid and antioxidant activity of green and red *Amaranthus* have already been reported in various studies. However, to our knowledge so far, there is no acceptable and reliable information on vitamin, phenolic, and flavonoid contents regarding the comparative analysis of red and green *Amaranthus* available in India and elsewhere.

MATERIAL AND METHODS

2.1 Plant material

Seeds of red and green *Amaranthus* were collected from local nursery. The seeds of red and green *Amaranthus* were sterilized using 0.2% of Bavistin to avoid fungal infection. The soaked grains seeds were sown in triplicates in pots having normal soil, soil with pomegranates peels, lemon peels, banana peels and pineapple peels.

Watering of pots was done regularly so that the soil remained moist. Sprouting and germination were carried out in an uncontrolled temperature (average 24-25°C). The microgreens were grown in the laboratory with daylight but no direct sunlight till day 7^{th}. The room was subjected to a diurnal cycle with fluctuations of natural temperature, humidity, and light.

2.2 Preparation of sample: The samples were harvested day 7th days after sowing and all the suspended dirt particles were thoroughly removed. Nearly 1gm of sample was weighed and washed. The microgrens were crushed using a mortar/ pestle and dissolved properly in 10ml of sterilised water to make the concoction.

2.3 Estimation of total phenol content (TPC): The total phenol content (TPC) was determined spectrophotometrically using tannic acid as a standard with some modifications **[3]**. 1.0ml of the diluted sample extract (in triplicate) was added to tubes containing 5.0ml of $^{1}/_{10}$ dilution of Folin-Ciocalteu's reagent in water. Then, 4.0ml of a sodium carbonate solution (7.5% $^{w}/_{v}$) was added and incubated at room temperature for one hour. The absorbance was measured at wavelength 765nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of tannic acid equivalent per g dry weight (mg TAE/g).

2.4 Determination of Total flavonoid content: Total flavonoid content was measured by the modified aluminium chloride colorimetric assay **[3]**. The reaction mixture consisted of 1.0ml of extract and 4ml of distilled water taken in a 10 ml volumetric flask. To the flask, 0.30ml of 5% sodium nitrite was added and after 5 minutes, 0.3 ml of 10% aluminium chloride was mixed. After 5 minutes, 2.0ml of 1M Sodium hydroxide was added and final volume of the mixture was brought to 10ml with double-distilled water. The absorbance for test and standard solutions were determined against the reagent blank at wavelength 510nm with an UV/Visible spectrophotometer. The total flavonoid content was calculated from the calibration curve and was expressed as mg Ascorbic acid equivalent (AAE)/g of extract.

2.5 Determination of antioxidant power by using modified ferric ion reducing antioxidant power assay (FRAP). The total antioxidant capacity was determined spectrophotometry, using ascorbic acid as standard and using the modified FRAP assay **[3]**. 0.1ml of extract was taken and to it 0.9 ml of ethanol, 5.0ml of distilled water, 1.5ml of HCl, 1.5ml of potassium ferricyanide, 0.5ml of 1% SDS and 0.5ml of 0.2% of ferric chloride was added. This mixture was boiled in water bath at 50°C for 20 minutes and cooled rapidly. Absorbance was measured at wavelength 750nm to measure the reducing power of the tea extract. The antioxidants in samples were derived from a standard curve of ascorbic acid and were expressed as mg ascorbic acid equivalent (AAE)/ g.

2.6 Estimation of ascorbic acid. Ascorbic acid was measured spectrophotometrically by 2, 4-DNPH method. 0.3ml of extracts was pipetted out in test tubes **[3]**. To all the test tubes containing extract, distilled water was added to make up to 1.5ml. To all the test tubes, 0.5 ml of 2, 4- DNPH was added and after proper mixing, test tubes were incubated at 37º C for 3 hours. 3.5ml of 80% H_2SO_4 was added to the test tubes to dissolve the orange red osazone crystals formed and absorbance was spectrophotometrically measured at wavelength 540nm.

2.7 Statistical Analysis:

The assays were carried out in triplicate, and the results were expressed as mean values and the standard deviation (SD). The statistical differences were done by one-way ANOVA ($p \leq 0.05$).

RESULT AND DISCUSSION

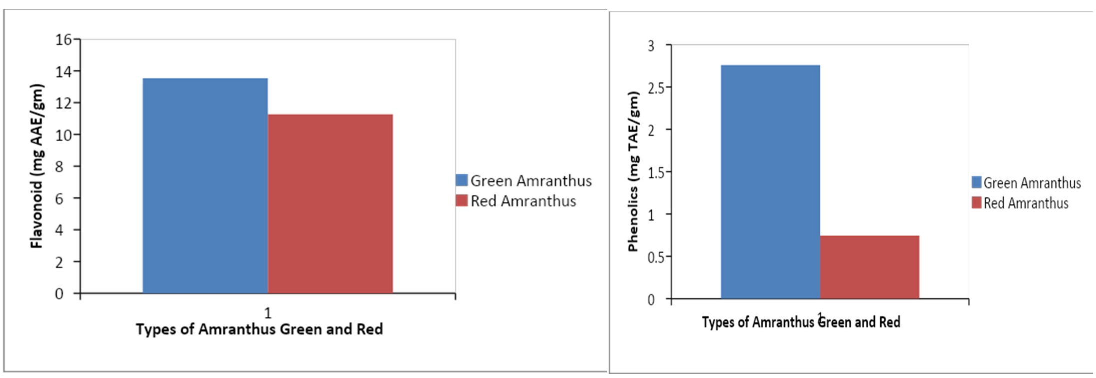
The germination rates were fastest in green than red *Amaranthus*. Seedling emergence occurred within 4 days in green but red *Amaranthus* took 6 days. Various other studies also reported difficulty in growing red *Amaranthus* seeds and recommended seed priming and application of plant growth regulators to break dormancy **[4, 5]**.

The carbohydrate levels were almost similar in green (4.57±0.05mg/ml) and red *Amaranthus* (4.49±0.15mg/ml). The *Amaranthus* contain 65% to 75% starch, 4% to 5% dietary fibres, and sucrose, glucose, fructose and nonstarch polysaccharide components **[6]**. Sucrose was the major sugar followed by raffinose, whereas inositol, stachyose, and maltose were found in small amounts.

The protein levels were significantly higher in red (0.020±0.0024mg/ml) than green *Amaranthus* (0.016±0.008mg/ml). The proteins present in *Amaranthus* contain high amounts of some essential amino acids e.g., leucine, lysine, sulphur amino acid, aromatic amino acids. It also contains high amounts of albumins, globulins, prolamins, and glutelins **[7]**. During germination, amounts of amino acids such as Aspartic acid, Serine, and Alanine increases. Thus, *Amaranthus* microgreens can also be as a good source for high-quality proteins are a valuable raw material for the preparation of protein concentrates (APCs) **[7]**.

The phenolics levels in green *Amaranthus* (2.76±0.124 mg TAE/gm) was statistically higher than red *Amaranthus* (0.745±0.074 mg TAE/gm) p(≤0.05). The various phenolic acids present in *Amaranthus* are salicylic acid, vanillic acid, protocatechuic acid, gallic acid, gentisic acid, β-resorcylic acid, *p*-hydroxybenzoic acid, syringic acid, ellagic acid, sinapic acids, chlorogenic acid, *trans*-cinnamic acid, *m*-coumaric acid, caffeic acid, *p*-coumaric acid. The chlorogenic acid, is one of the major phenolic found in *Amaranthus* microgreens, and is found to be most efficient and abundant antioxidant products of the phenylpropanoid pathway in young plant tissues **[8, 9]**. However, at 30th day of *Amaranthus* growth, the phenolic levels in the red genotype were higher as compared to green *Amaranthus* as reported **[1]**. They observed higher levels of salicylic acid, vanillic acid, gallic acid, *p*-hydroxybenzoic acid, syringic acid, ellagic acid, chlorogenic acid, *m*-coumaric acid, caffeic acid, *p*-coumaric acid, ferulic acid, rutin and isoquercetin in red than green *Amaranthus.*

The flavonoid levels in green (13.53±0.089mg AAE/gm) was statistically higher than red *Amaranthus* (11.9±0.0690 mg AAE/gm) p(≤0.05). The flavonoids such as rutin, hyperoside, isoquercetin, myricetin, quercetin, apigenin, kaempferol, and catechin are present in *Amarantus* as reported **[1]**. (Sarker and Oba, 2019). The phenolic levels in the red genotype were higher as compared to green *Amaranthus* at 30th day of germination. The red *Amaranthus* has highest isoquercetin, myricetin, quercetin, apigenin, kaempferol, catechin, and hyperoside whereas green *Amaranthus* has low levels isoquercetin, myricetin, quercetin, apigenin, kaempferol, catechin, and hyperside.



(a) **(b)**

Figure 1: The total flavonoid (a) and phenolic levels (b) in red and green Amaranthus after 7th day of germination

Vitamin C or ascorbic acid is an essential nutrient for the human body—it is required for the biosynthesis of collagen, carnitine and neurotransmitters **[10]**. Health benefits attributed to vitamin C include antioxidant, anti-aetherogenic, anti-carcinogenic, and immunomodulator effects. The vitamin C content of green *Amaranthus* sprouts (3.03±0.0103mg/ml) was statistically higher than red *Amaranthus* from red (2.83±0.0213).

The antioxidant level in green (2.64±0.0105mgAAE/gm) was statistically higher than red Amaranthus (2.397±0.0214). Various studies have reported that *Amaranthus* have antioxidant activity which could be due to vitamins present in Amaranthus *along* with carotenoids, flavonoids, and phenolic acids, lipophilic antioxidants such as squalene and tocols. The high amount of betacyanin in, which gives its deep red hue enhances antioxidant activity in red *Amaranthus* along with phenolic compounds **[11]**.

The total phenolic content, flavonoid content and antioxidant levels of red and green *Amaranthus* microgreens is affected by days of growth. In our study the increased phenolics, flavonoid and antioxidant levels in microgreen in green than red *Amaranthus* at 7th day of growth as compared to increased levels in red Amaranthus at 30th day of growth as observed in other study could be due to delay in germination of red germination **[1]**. Thus, it can be concluded that Am*aranthus* microgreen can be potential source of balanced diet for developing nations as recommended by the Food and Agriculture Organization of the United Nations and World Health Organization.

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