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THE PHENOLIC, FLAVONOID AND ANTIOXIDANT EVALUATION OF WHEAT (*TRITICUM AESTIVUM)* AND BARLEY (*HORDEUM VULGARE)* MICROGREEN

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ABSTRACT

Wheat and barley micro greens are emerging class of food that grows in a short time but have high nutritional value. These microgreens are associated with bioactive molecules like pigments, ascorbic acid, and polyphenols and eventually a high antioxidant activity. The total phenolic content, ascorbic acid and antioxidant levels were evaluated and compared in wheat and barley microgreens. In the present study the total phenolic content (TPC) was higher numerically in wheat as compared to barley microgreens on day 15^{th} and 17^{th}. The ascorbic acid was higher numerically in barley microgreen on day 15^{th} and 17^{th} as compared to wheat microgreen (0.11 ± 0.0152 mg/ml to 0.27633 ± 0.0111 mg/ml). The antioxidant levels in barley was significantly higher as compared to wheat microgreen in normal as well as in soil fortified with lemon, pomegranate, pineapple and banana peels on day 15^{th} and 17^{th} ($p \leq 0.05$)

Keywords: Microgreens, barley grass, wheatgrass, phytochemicals

1. INTRODUCTION

The Microgreens are an emerging class of produce which can change nutrient balance intake in a natural way. The use of young plantlets, 1–2 weeks of age, commonly termed as *microgreens* is increasing, because of their high concentrations of nutrients and health benefits **[1]**. Wheat (*Triticum aestivum)* and barley (*Hordeum* *vulgare)* belonging to the Poaceae family are important cereal in the northern plain in India. Wheat and barley microgreen are rich in amino acids and mineral salts (Ca, Mg, Fe, Mn, Zn, Se, and Mo), as well as secondary metabolites, such as polyphenols, anthocyanins, carotenoids and ascorbic acid in higher concentrations compared to mature plants.

Wheat and barley microgreen exhibit antioxidant properties which reduces the risk of bacterial infection **[2]**. These microgreens can play a great role in the treatment of health problems such as antidepressants, anti-ulcer, anti-oxidant, anti-diabetic, anti-inflammatory effects. These are also used for increasing production of haemoglobin, preventing tooth decay, improving wound healing and preventing bacterial infection.

The healthful properties of wheat and barley microgreen are largely attributed to polyphenol chemicals. Phenolic compounds exhibit antioxidant activity by inactivation of lipid free radical or hydrogen peroxide into free radicals such as superoxide radical, hydrogen peroxide, hypochlorous acid. Antioxidants such as polyphenols neutralise the free radical which attack the biological cell and may reduce or even help to prevent some of the damage they cause. These polyphenols contain free radical scavengers which help in preventing stress induced diseases such as cardiac disorders, diabetes mellitus, inflammatory, neurodegenerative diseases and cancer **[2]**. The wheat Barley microgreens also contain antioxidant enzymes SOD (superoxide dismutase) and cytochrome oxidase that has the potential to convert reactive oxygen species to hydrogen peroxide and an oxygen molecule.

These microgreens are rich in flavonoids which exhibit several biological effects such as anti-inflammation, anti- ulcer actions, anti-viral, anti- allergic. They are potent antioxidants and have free radical scavenging ability **[3]**. Flavonoids have been shown to be highly scavengers of most types of oxidizing molecules, including singlet oxygen, other free radicals **[4]**.

Wheat and barley microgreen are rich in dietary minerals such as sodium, magnesium, iron, copper and phosphorous and vitamins. They are rich in pigments such as chlorophyll and carotenoids. Chlorophyll plays an important role in neutralizing toxins in the body and help to purify the liver. Microgreens contain twenty amino acids involved in energy production and cell building and regeneration **[5]**. Consumption of barley and wheat microgreen has the potential to lower the levels of blood cholesterol, a risk factor for cardiovascular diseases and also improves regulation of blood sugar.

In the present study wheat and barley microgreen grown in soil which was mixed with different fruit peel powder (lemon, banana, pineapple, pomegranate, mosambi). Fruit peels waste is an ideal source of natural antimicrobials. Fruit peel is a good source of several nutrients such as fibres, vitamins, minerals, anti-oxidants and phenolic compounds that have strong potentials for antitumor, antibacterial, antiviral, cardio protective and anti-mutagenic. Wheat and barley seeds were sown in the prepared soil and after its growth the extract was obtained and the antioxidant assays were performed.

2. MATERIAL AND METHODS

2.1 Plant material

Seeds of wheat and barley were collected from a local mill and fruit peels of lemon, banana, pomegranate, pineapple and mosambi (sweet lemon) were collected separately from local fruit juice shop. Peels of banana, pomegranate, pineapple and lemon were sun dried and then dried in a hot air oven at 70 degrees for 3-5 days. The dried fruit peels were powdered and stored at room temperature. 2g peel powder was added to a 100g homogenous mixture of soil. The grains of wheat and barley 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 grasses were grown in the laboratory with daylight but no direct sunlight till day 17^{th}. The room was subjected to a diurnal cycle with fluctuations of natural temperature, humidity, and light.

2.2 Preparation of sample: The sample was harvested 1 cm above the soil on days 15^{th} and 17^{th} days after sowing and all the suspended dirt particles were thoroughly removed. Nearly 1gm of sample was weighed and washed. The leaves 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 **[6]**. 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 765nm wavelength. 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 **[6]**. 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 the 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 by spectrophotometry, using ascorbic acid as standard and using the modified FRAP assay **[6]**. 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 a 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 **[6]**. 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 ($p \leq 0.05$).

3. RESULTS and DISCUSSION

In the present study the total phenolic content (TPC) were higher numerically in wheat (0.15267 ± 0.0709 mg TAE/g to 0.279 ± 0.01 mg TAE/g) as compared to barley microgreens (0.11 ± 0.0152 mg TAE/g to 0.27633 ± 0.0111 mg TAE/g) on day 15th in normal as well as in soil fortified with lemon, pomegranate, pineapple and banana peels **(Figure 1)**. On day 17th also, the phenolic levels were higher in wheat as compared to barley microgreen. The difference in total phenolic content between two species could be due to the difference in nutritional composition of cereals **[7]**. However, statistically no significant difference was found in TPC levels on 15th and 17th day in wheat and barley microgreens grown in all types of soil. The results showed that maximum TPC (0.34033 ± 0.0153 mg TAE/g) was found in wheatgrass grown in soil fortified with pineapple peels on day 17th , while the minimum TPC (0.11 ± 0.0152 mg TAE/g) was reported in barley grass on day 15th in soil fortified with banana peels. This variation in TPC could be due to differences in nutrient levels in grasses which are maximum at jointing stage and decrease with the development of non-phenolic structural components like cellulose and hemicellulose in leaves, leaf veins, stems, and nodes **[8]**. The increase in TPCs in microgreens was probably due to the stimulation of phenylpropanoid pathways **[9]**. Various studies have reported that TPCs levels in wheat grass reached a maximum level earlier than barley grass as observed in this study **[7, 8]**. The phenolics levels were higher but not significantly in wheat microgreen grown in normal as well as soil with fruit peels on 17th day as compared to 15th day. However, in barley the phenolics levels were significantly higher when grown in soil with pomegranate and pineapple peels on day 17th as compared to day 15th. The delayed increase in TPC levels of barley could be due to a delayed growth rate. The bioavailability of minerals in soil such as K^{+}, Ca^{++}, Na^{+}, Fe^{+++}, P, and Mg^{++}, present in fruit peels might have contributed to enhanced levels of phenolics **[10]**.

In the present study, the ascorbic acid was higher numerically in barley grass on 15th and 17th day as compared to wheat microgreen. The higher level of ascorbic acid in barley microgreen was reported earlier also **(Figure 2) [11]**. The ascorbic levels were similar on day 15th and 17th in wheatgrass as reported earlier **[10]**. However, the ascorbic acid levels were significantly higher on day 17th as compared to day 15th in barley grass grown in normal soil and soil fortified with fruit peels. The increase in ascorbic acid content in barley microgreens till day 17th could be associated with the reactivation of enzyme L-galactono-1,4-lactone dehydrogenase, which catalyzes the last enzymatic step of ascorbic acid biosynthesis. However, in wheatgrass, constant levels of ascorbic acid could be due to the onset of the conversion of ascorbic acid to tartarate in the presence of L-idonate dehydrogenase, or C2/C3 cleavage into oxalate and L-threonate leading to its reduction in subsequent days **[10, 12]**.

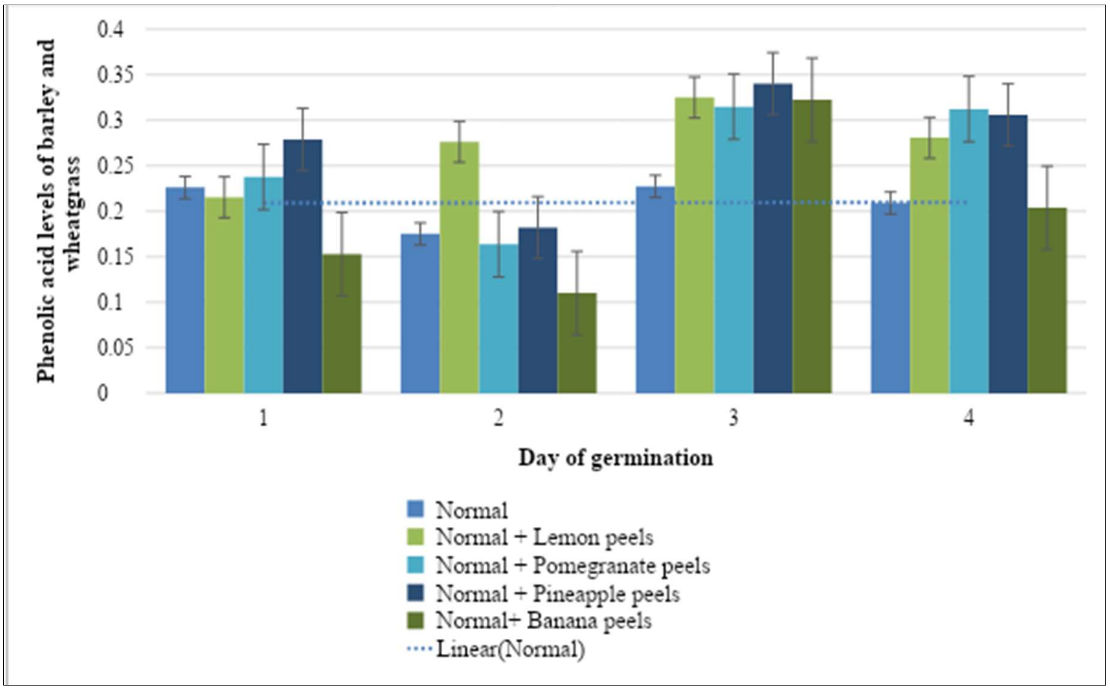

Figure 1: The total phenolic levels of wheat and barley microgreen in different types of soil after 15th and 17th day of germination

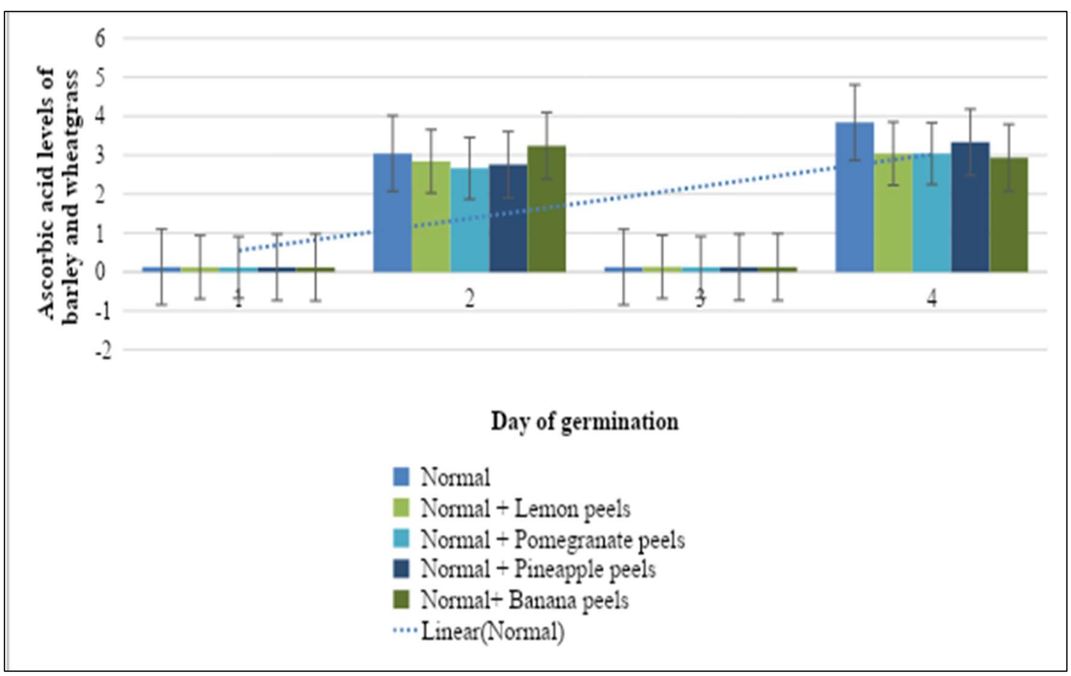

Figure 2: The ascorbic acid levels of wheat and barley microgreen grown in different types of soil after 15th and 17th day of germination

The antioxidant levels in wheat microgreen ranged from 2.663±0.0213 mg AAE/gm to 3.23967±0.0105 mg AAE/gm whereas in barley it ranged from (3.39±0.07 mg AAE/gm to 3.933±0.060 mg AAE/gm) on day 15th. On day 17th, the antioxidants range from 2.379±0.0819 mg AAE/gm to 2.70633±0.36 mg AAE/gm in wheat microgreen and 3.2466±0.0152 mg AAE/gm to 4.42667± 0.0321 mg AAE/gm in barley microgreen. Thus, the antioxidant levels in barley was significantly higher as compared to wheat microgreen in normal as well as in soil fortified with lemon, pomegranate, pineapple and banana peels on day 15th and 17th ($p \leq 0.05$). The significantly higher antioxidants in barley than wheat microgreen was reported earlier **[8]**. This wide variation in wheat and barley microgreen could be due to the nutritional composition of cereals which varies depending on their botanical origin **[7]**. Thus, wheat and barley microgreen can be great sources of natural antioxidants as they provide better protection against lipid peroxidation and thereby decreased oxidative stress and increased endogenous antioxidant levels such as plasma total antioxidant status and vitamin C **[14]**. The antioxidants levels remained almost constant in wheat microgreen grown in soil fortified with lemon peels, pomegranate and pineapple peels on day 17th as compared to day 15th. In barley microgreen, the antioxidants levels remained almost constant in grown in soil fortified with fruit peels from day 15th to 17th. However, there was significant reduction in antioxidant level in normal soil in both wheat and barley on day 17th. Thus, it can be inferred that bioactive compounds present in fruit peels contribute to antioxidant levels of microgreens.

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