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Research Article

PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY MODIFICATIONS IN WHEATGRASS (*TRITICUM AESTIVUM* L) CULTIVATED IN ORGANICALLY FORTIFIED SOIL WITH FRUIT PEELS

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ABSTRACT

Wheatgrass (*Triticum aestivum* L) is an important source of dietary supplement such as chlorophyll, amino acids, minerals, vitamins, and enzymes and antioxidant compounds, such as carotenoids, tocopherols, tocotrienols, phenolic acids, phytic acid, phytosterols and flavonoids. The bioactive compounds present in wheatgrass are influenced by various factors, such as water availability, soil composition fertilization regimes and light quality. The effects of wheatgrass length and fruit peel based organic fertilizer on the phytochemical constituents and antioxidant potential of grass juices was evaluated on day 15th, 17th, 22nd, 24th and 26th of germination of wheat. The highest antioxidant levels were observed on day 15 of growth under condition of soil treated with banana peels (3.23967±0.0105 mg AAE/gm). However, the values of total flavonoids (0.71733±0.089 mg AAE/gm) and ascorbic acid (0.157±0.01 mg/ml) were highest on 26th day in wheatgrass grown in soil formulated with mosambi peels. The total phenolics were highest on day 17th in wheatgrass grown in soil formulated with pineapple peels (0.34033±0.0153 mg TAE/gm). The present study concludes that bioactive compounds present in the fruit peels contribute in enhancing the antioxidants levels of wheat grass juice.

Keywords: Antioxidant, Flavonoid, Phenolic, Ascorbic acid, Wheatgrass.

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) germinated over a period of 6-10 days is generally called 'wheatgrass'. Wheatgrass is a rich source of vitamins C and E, β-carotene (vitamin A), ferulic acid and vanillic acid antioxidants whose concentration increases with the germination period [1].

Wheatgrass juice is known as a superfood because of various benefits for human health [2]. It is reported that wheatgrass has shown anticancer activity [3], antiulcer activity [4], anti-inflammatory [5], antioxidant activity [3], antiarthritic activity [6], antimicrobial activity [7] and a low-density lipoprotein (LDL) lowering effect [8]. Wheatgrass juice produce red blood cells and can be used as an adjuvant in the treatment of conditions such as thalassemia [9]. These positive effects are due to the high level of chlorophyll content which inhibits the metabolic activation of carcinogens [10].

Wheatgrass juice has been traditionally consumed as an herbal medicine which is known to increase the strength of immune system. There is a need to improve wheatgrass quality by altering cultivation parameters, in order to obtain higher quality wheatgrass with enhanced phenolic contents and antioxidant level to be used as natural antioxidant supplements. Various reports suggest that fruit peels as can be used as fertilizer as they regulate the pH of the soil and also helps in supplementing soil with various micronutrients like iron, calcium and zinc. Fruit peels are rich source of dietary proteins, amino acids (*i.e.,* L-tryptophan), nutrient elements such as potassium, and growth promoters [11]. The effect of different fruit peels on the soil condition used for the cultivation of wheatgrass has not been studied extensively. To fulfil these lacunae, the present study assessed the bioactive constituents and antioxidant level in aqueous extracts of the wheatgrass germinated over a period of 15th, 17th, 22nd, 24th and 26th day.

2. MATERIAL AND METHODS

2.1. Plant material

Seeds of *Triticum aestivum* L (variety - MP sharbati) 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 sundried and then dried in a hot air oven at 70°C for 3-5 days. The dried fruit peels were powdered and stored at room temperature. Two (2) g Peel powder was added to 100g soil. Seeds of *Hordeum vulgare* L. were sterilized using 0.2% of Bavistin to avoid fungal infection. The seeds were sown in triplicates in pots having normal soil, soil with pomegranates peels, lemon peels, banana peels and pineapple peels. The wheatgrass was grown with the fruit peels formulated soil in small pots. The grass was cut when it reached at a height of nearly 10-12 inches long (pre-jointing stage).

2.2. Preparation of sample wheatgrass extract

The fresh wheatgrass from each soil sample was cut at the first node on 15^{th}, 17^{th}, 22^{nd}, 24^{th} and 26^{th} day of germination and all the suspended dirt particles were thoroughly removed. Nearly 1gm of fresh green leaves were 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 [5]. One ml 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 [5]. 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 anti-oxidant power assay (FRAP):

The total antioxidant capacity was determined spectrophotometry, using ascorbic acid as standard and using the modified FRAP assay 5. 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 were pipetted out in test tubes [5]. 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 37°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 ($p \leq 0.05$).

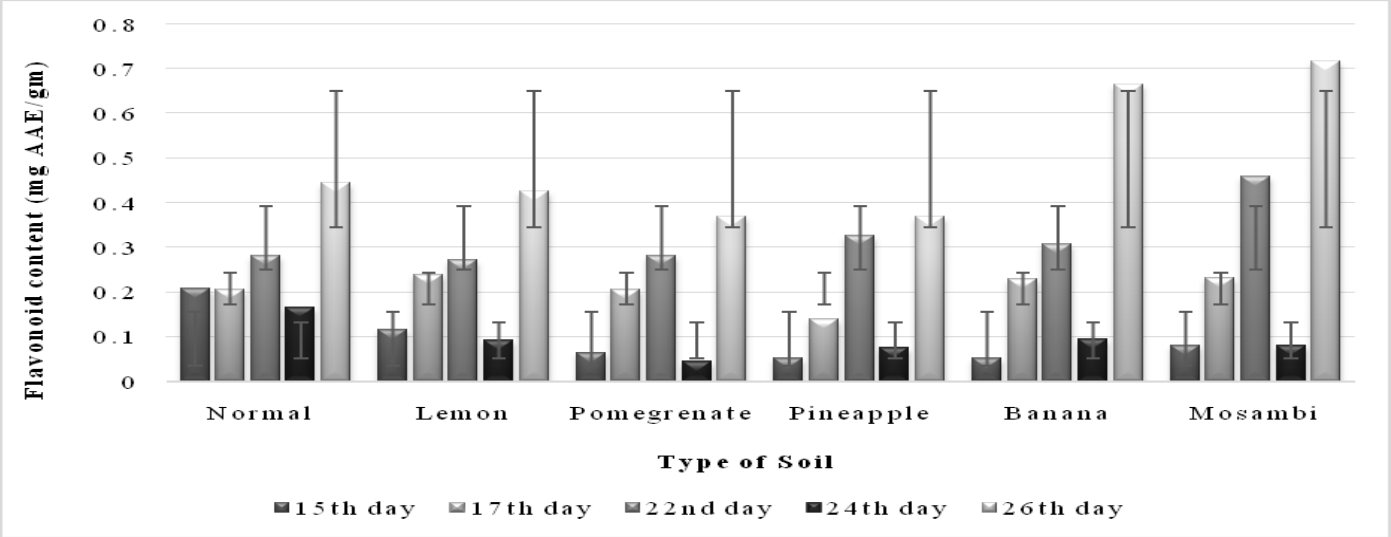
3. RESULTS AND DISCUSSION

In wheatgrass grown in normal soil, the flavonoid levels remained constant followed by significant increase in flavonoid level on day 22^{nd} and 26^{th} as compared to day 17^{th} and 24^{th} ($p \leq 0.05$) followed by significant decrease on day 22^{nd} ($p \leq 0.05$). However, there was significant uptrend in flavonoid levels on day 26^{th} as compared to day 15^{th}, 17^{th}, 22^{nd} and 24^{th} ($p \leq 0.05$). In wheatgrass grown in soil treated with pomegranate, banana, pineapple, and lemon peels the flavonoid levels showed an upward trend from day 15^{th} to 26^{th} except on day 24^{th} where there was significant decrease in the flavonoid levels ($p \leq 0.05$). In all the cases, the flavonoid levels showed a significant increase on day 26th as compared to

day 24th (p≤0.05). The highest flavonoid levels were observed in mosambi (0.71733±0.089 mg AAE/gm) followed by banana peels (0.66533±0.030 mg AAE/gm) on day 26th One of the most important flavonoids present in wheatgrass juice is apigenin, which prevents the transactivation induced by TNF-α. Apigenin also inhibits inflammasome pathways and production of IL-1β resulting in down-regulation of iNOS and COX-2 [12].

In wheatgrass grown in normal soil, the total phenolics content (TPC) remained constant till day 24th followed by significant increase in phenolics level on day 26th (p≤0.05). In wheatgrass grown in soil treated with lemon, banana, pineapple, mosambi peels, the phenolics levels showed a significant increase on day 17th and 24th as compared to day 15th and 22nd (p≤0.05). The highest phenolic levels were observed on day 17th in pineapple (0.34033±0.0153 mg TAE/ gm) followed by banana (0.32233±0.024 mg TAE/ gm), lemon (0.325±0.02 mg TAE/gm) and pomegranate peels (0.315±0.01 mg TAE/gm). The major phenolics present in wheatgrass are benzoic acid, caffeic acid, galic acid, syringic acid, p-hydroxybenzoic acid, ferulic acid. sinapic acid and ferulic acid content was observed to be closely related to radical scavenging capacity. The increase in TPC could be due to production of phenolic compounds by plants through the phenylpropanoid pathway so as to prevent seedling from damages caused by environmental stresses during early growth [13]. The bioavailability of minerals in soil such as K^+, Ca^{++}, Na^+, Fe^{+++}, P and Mg^{++}, present in fruit peels might have contributed to enhanced functioning of some enzymes of the phenylpropanoid and flavonoid biosynthetic pathways such as phenylalanine ammonium lyase, CoA-ligase or methyltransferases.

In wheatgrass grown in normal soil, lemon peels, pomegranate, pineapple, banana and mosambi, the vitamin C levels remained constant from day 15th to 17th followed by significant constant decrease in vitamin C level on day 22nd and 24th followed by significant increase on day 26th (p≤0.05). The highest vitamin C value was recorded on day 26th in mosambi (0.157±0.01 mg/ ml) followed by banana (0.1465±0.015 mg/ml), lemon (0.14267±0.020 mg/ml) and pomegranate peels (0.13633±0.0153 mg/ml).The increase in the level of ascorbic level could be attributed to enhanced levels of L-galactono-gamma-lactone dehydrogenase (GLDH) enzyme involved in ascorbic acid biosynthesis by catalysing the oxidation of L-galactono-1,4-lactone to ascorbic acid in germinating seeds [14]. Since citrus peels are rich in flavonoids and vitamin C which might have contributed to increased ascorbic acid levels in wheatgrass juice.

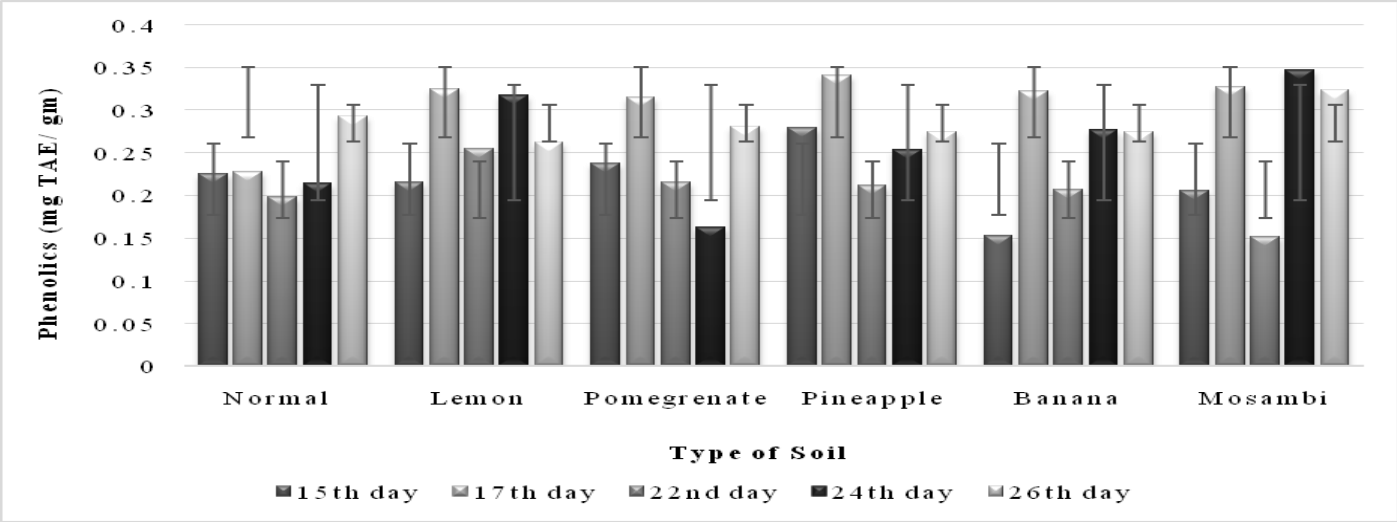


Normal soil, soil with lemon peels, soil with pomegranate peels, soil with pineapple peels, soil with banana peels and soil with mosambi peels on various days (15th, 17th, 22nd, 24th and 26th day).

Fig. 1: Changes of flavonoid activities in wheatgrass grown in different conditions

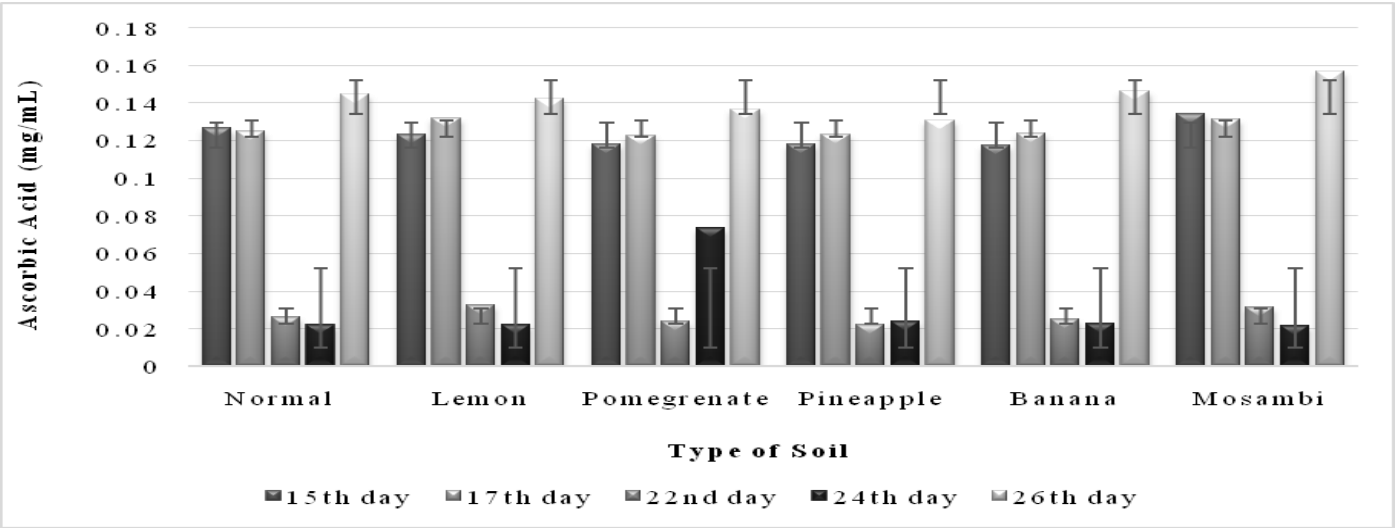
Antioxidant activity evaluation during wheat grass development was done on 15th, 17th, 22nd, 24th and 26th day. Then, there was a significant time-dependent decrease in antioxidant activity until it reached the lowest value on 26th day in normal soil (1.52733± 0.0264 mg AAE/ gm) and soil with pomegranate

(1.93367±0.020 mg AAE/ gm) and pineapple peels (1.3393±0.0550 mg AAE/ gm). In wheat grass grown on soil with lemon peels, the antioxidant levels remained constant from day 15th to day 17th same followed by decreased antioxidant activities on day 22nd, 24th, 26th ($p \leq 0.05$). In soil treated with banana peels, the antioxidant levels have the highest value on day 15th (3.23967±0.0105 mg AAE/gm) which drastically decrease through day 17th, 22nd and 24th to reach its lowest value on day 26th (1.40567±0.0321 mg AAE/ gm). In all the cases there was drastically dip in antioxidant levels on day 24th. The antioxidant action of wheat grass is due to presence of bioflavonoids like apigenin, quercitin and luteolin and other compounds such as indole compounds, choline and laetrile (amygdalin). The bioflavonoids such as apigenin which are a part of xanthine/xanthine oxidase pathway and enzymes such as Super oxide dismutase (SOD) contributes to the radical scavenging capacity of WP by converting free radical reactive oxygen species (ROS) into hydrogen peroxides [15]. Various other compounds such as chlorophyll, ascorbic acid also synergize with phenolic compounds and may contribute to higher antioxidant activities of the wheatgrass juice [2].



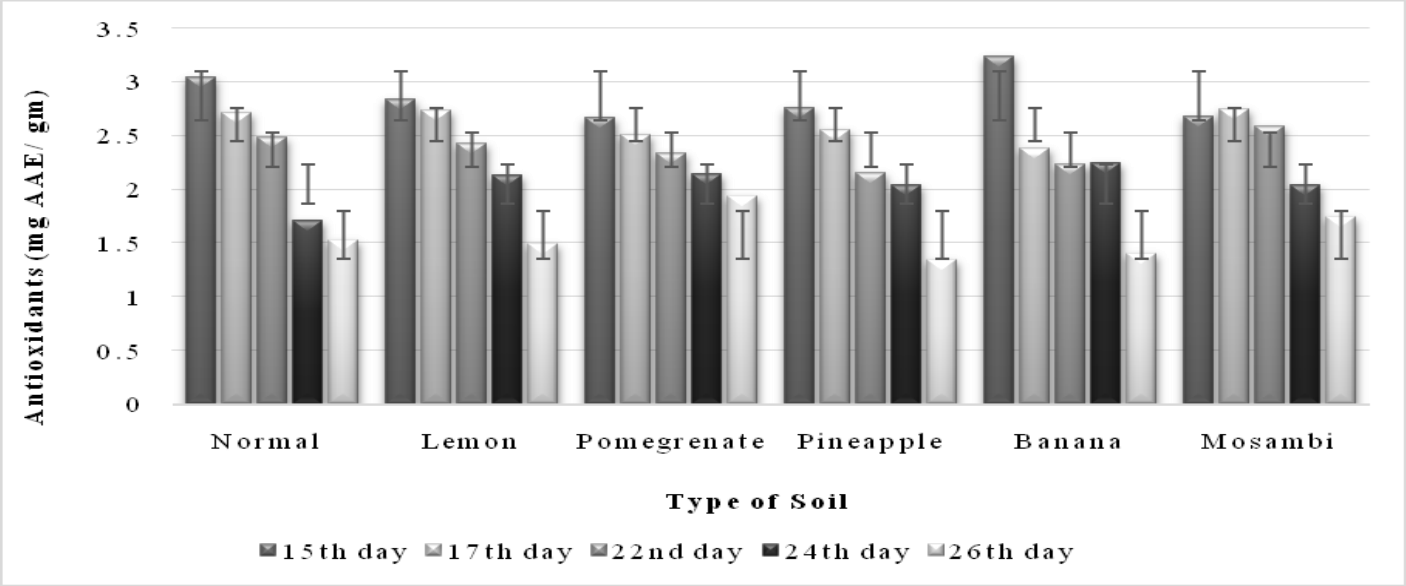
Normal soil, soil with lemon peels, soil with pomegranate peels, soil with pineapple peels, soil with banana peels and soil with mosambi peels on various days (15th, 17th, 22nd, 24th and 26th day).

Fig. 2: Changes of phenolic activities in wheat grass grown in different conditions



Normal soil, soil with lemon peels, soil with pomegranate peels, soil with pineapple peels, soil with banana peels and soil with mosambi peels on various days (15th, 17th, 22nd, 24th and 26th day).

Fig. 3: Changes of vitamin C activities in wheat grass grown in different conditions



Normal soil, soil with lemon peels, soil with pomegranate peels, soil with pineapple peels, soil with banana peels and soil with mosambi peels on various days (15th, 17th, 22nd, 24th and 26th day).

Fig 4: Changes of antioxidant activities in wheat grass grown in different conditions

4. CONCLUSION

The results suggest that antioxidant activity of wheatgrass juice can be enhanced by fruit peels enriched soil. The increased antioxidant capacity of wheatgrass juice can be manifested in the form of increased natural ability of wheatgrass to counter the excessive radicals. However, further investigations need to be done to establish optimum nutrient levels and availability of bioactive compounds for use of wheatgrass as 'functional food'.

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