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Chemical Composition, Minerals and Vitamins Analysis of Lyophilized Wheatgrass Juice Powder

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ABSTRACT: Wheatgrass is known as significant source of vitamins, minerals, carbohydrates, enzymes, chlorophyll and polyphenols. Lyophilization technique helped to preserve the heat sensitive constituents and enhanced shelf-life of product. The lyophilized wheatgrass juice powder subjected to use for chemical, nutritional and antioxidant analysis. Chlorophyll, the main constituent of wheatgrass was analyzed as 7.46 mg/g and essential elements such as Fe, Mg, Zn, K and Mn having higher concentration in wheatgrass juice powder observed by AAS. The presence of vitamins B1, B2, B3, B4, B6, B10 and C detected at 254nm by following RP-HPLC-PDA method and their assistance to enhance antioxidant potential determined with DPPH assay. The presence of these amazing phytoconstituents in wheatgrass makes it one of the healthiest remedy to cure minor ailments to chronic disorders.

Keywords: Lyophilization, wheatgrass, chlorophyll, vitamins, minerals, anti-oxidant.

I. INTRODUCTION

Cereal grasses have great importance in various cultures of world because of health saving benefits for all wellbeing. Wheatgrass, one of them has played important role as dietary supplement in most of the countries. The 7-10 days germinated wheat seedlings are called as wheatgrass that recognized as detoxifying agent which maintained the alkalinity of blood. Wheatgrass enriched with chlorophyll content that actively suppressed the metabolic functioning of carcinogenic compounds [1]. The presence of polyphenols, vitamins like β-carotene, ascorbic acid and chlorophyll pigments revealed the antioxidant potentiality i.e. high at sprouting stage [2]. Different changes observed during germination period due to the impact of physical parameters. The beneficial primary and secondary metabolites such as minerals, vitamins, polyphenolic and antioxidant compounds have been synthesized in wheatgrass. Varying proportions of K/Ca caused alteration in anti-oxidative mechanism of phytoconstituents. Elevating concentration of K enhanced the polyphenolic components and vitamins that improved the quality of product [3]. Schnabel recognized as pioneer of wheatgrass who familiarized it as beneficial medicinal therapy for human beings. According to him the nutritive quality of cereal grasses depended on their developmental and growth levels [4]. Daily intake of wheatgrass reduced the risks of chemotherapy and need of medicines in breast cancer suffering patients [5]. The presence of poly-phenols in wheatgrass assisted to revert the consequences of reactive oxygen species and limited the chances of cancer like disease [6]. Due to increasing demand of antioxidant rich food, the wheatgrass became familiar to all and available in different forms like drinks, capsules and powder. Such kind of antioxidant rich products have anti-ageing properties and protect us from chronic diseases like cancer, Alzheimer [7]. The balanced diet enriched with minerals, vitamins and proteins needed to regulate the body functioning in adequate manner. Lack of any nutrient lead toward malfunctioning of organs and caused chronic diseases. The wheatgrass known as complete nutrient package that enclosed important minerals such as magnesium, zinc, selenium and vitamins like vitamin A, E, C and polyphenols like ferulic acid, cinnamic and gallic acid etc., [8]. The presence of flavonoids and apigenin like inflammation reductants were assisted to reduce the severe abdominal pain and rectal bleeding in case of ulcerative colitis disease [9]. Our study based on analysis of active constituents or metabolites such as vitamins, minerals and antioxidant by following methods of HPLC (High Performance Liquid Chromatography), AAS (Atomic Absorption Spectroscopy) and spectrophotometer precisely.

II. MATERIAL AND METHODS

A. Material

Wheat grains (*Triticum aestivum* L.) of best quality were taken from local market for experimentation and surface sterilized by using 0.1% NaOCl. After proper washing of wheat grains with distilled water, cultivation was carried out in plastic trays (Dimensions: length-49.0 cm, width-28.0 cm and depth-2.5 cm) at laboratory scale under controlled temperature and light conditions. The salts of Hi Media and analytical grade solvents were utilized for analysis.

B. Wheatgrass Juice Extraction and Lyophilization

On tenth day wheatgrass was cut with sterilized knife and after washing immediately took for extracting juice.

Extracted juice was preserved into deep freezer and then lyophilized by using Martin Christ-Lyophilization unit. The process carried out in three phases i.e., freezing, primary drying and secondary drying. The lyophilized Wheatgrass Juice Powder (WGJP) was further used for experimental studies.

C. Pesticide Analysis

The GC-MS based pesticide analysis of wheatgrass juice powder was accomplished by using GC-MS (Thermo Finnegan) Trace GC ultra and Polaris Q equipment. The oven temperature of GC was initially held at 80℃ for 1 minute, then 200℃ @ 25℃/min for 2 minute to 230℃ @ 2℃/min for 1 minute and finally its 280℃ @ 2℃/min for 10 minutes. The PTV-LV (Programmable temperature vaporizing large volume injector) temperature was 50℃ and 40-450 amu acquired mass range along with helium as a carrier gas.

D. Proximate Composition Analysis

The pH, moisture and ash content of WGJP (Wheatgrass juice powder) were analyzed by following AOAC methods [10]. Acidity, crude fat, crude protein and crude fiber were determined by methods of Ranganna [11] and total soluble solids (TSS) analyzed by using Abbe's refractometer [12]. Anthrone assay used to analyze carbohydrates [13], phenol-sulphuric acid method for total sugar content [14] and DNSA (3, 5-Dinitrosalicylic acid) method for reducing sugars [15] by observing color intensity at absorbance (Abs) 630 nm, 490 nm and 575 nm spectrophotometrically. Carbohydrate and sugar content analyzed as GluE (glucose equivalent) mg/g dry weight of extract by using calibration curve of standard glucose.

E. Chlorophyll and Carotenoid Estimation

Chlorophyll and carotenoid content was estimated by following the method of Arnon with slight modifications [16]. The 100mg of powdered sample was dissolved in 80% acetone and sonicated properly. After sonication supernatant was filtered through Whatmann filter paper no. 1. Repeated the procedure 2-3 times and made final volume 10 mL. Then the absorbance of supernatants was determined at 663 nm, 645 nm and 470 nm by using UV/Visible spectrophotometer. The amount of pigments was determined by using following formulas:

$$\text{Chlorophyll a (mg/g)} = (12.7 \times A_{663}) - (2.69 \times A_{645})/w \times V$$

$$\text{Chlorophyll b (mg/g)} = (22.9 \times A_{645}) - (4.68 \times A_{663})/w \times V$$

Total Chlorophyll = Chl a + Chl b

Carotenoids + Xanthophyll (mg/g) = 1000 A470 – 1.90Chl a – 63.14 Chl b /214

where, V is the total volume in which sample dissolved and w is the weight of fresh powdered sample used for chlorophyll estimation.

F. DPPH Radical Scavenging Assay

The DPPH free radical scavenging capacity of extracts was analyzed by using procedure of Blois, with slight modifications [17]. The DPPH (2, 2-diphenyl-2-picryl hydrazyl) act as a stable free radical which changed to diphenylpicryl hydrazine when interacted with an antioxidant. Its color change from dark violet to yellowish determined at 517nm spectrophotometrically.

The extent of discolorations showed the antioxidant ability and hydrogen ion donating capacity of extract. Freshly formulated 2mL DPPH solution was added to the 1mL extract at different concentrations and incubated in dark for 30 minutes at room temperature. After incubation absorbance was Staken at 517nm by using ascorbic acid as standard. The DPPH solution was prepared in 95% ethanol. The antioxidant potential of extracts determined by using following formula:

$$\text{DPPH radical scavenging activity (\%age)} = \{(A_{Control} - A_{Sample}) / (A_{Control})\} \times 100$$

The $A_{Control}$ indicated the absorbance of ethanol + DPPH whereas A_{Sample} is the absorbance of plant extract + DPPH.

G. Elemental Analysis

The beneficiary elements were analyzed with the help of AAS (Atomic Absorption Spectrometer of Agilent Technology). The 500 mg powdered sample was digested on digesting unit by using HNO_3 and HF (4:2) as digesting chemicals. After that volume of digestive samples were prepared up to 50mL by using double distilled water for elemental analysis. Stock solutions of desired mineral standards were prepared in the range of 0.5 ppm to 50 ppm for calibration and then analyzed the samples after filtration [18].

H. Vitamin Analysis by HPLC

Vitamin B-complex (B1, B2, B3, B4, B6 and B10) and vitamin C were analyzed by RP-HPLC-PDA (Reversed phase-high pressure liquid chromatography with photodiode array detector) method in various extracts.

Chemicals Used: The HPLC grade solvents such as acetonitrile, methanol and water were acquired from Merck, Life Science Pvt. Ltd. Mumbai, India and the standards of vitamins from Sigma-Aldrich, USA.

Sample Preparation: Constituents are extracted from sample by sonication using methanol: water (80:20) as extracting solvents. The filtered extracts were dried by using rotary vacuum evaporator at 45℃. Now dried extracts were immersed in methanol (HPLC grade) and filtered by 0.22 µm filter. Different concentrations of samples were prepared for estimation. Stock solutions of vitamin standards were prepared as 1.0mg/2mL in methanol: water (50:50 v/v) solvents. For making standard curve concentration range of 1.0µg/mL to 5.0µg/mL was taken.

HPLC Instrumentation and experimental conditions: The HPLC based vitamin analysis of extracts was accomplished by Waters Semi Prep HPLC system equipped with Waters 2998 Photodiode Array Detector (PDA), 1525 binary pump and temperature controller module-II column oven. Samples solutions were filtered by 0.22µm filters (Millipore, India) and analysis carried out through Waters 2707 auto sampler and Waters Empower 3 software. Vitamin separation was carried on Luna C18-(2) 100A (250mm × 4.6mm, 5.0µm particle size) column of Phenomenex® (USA) by using acetonitrile: water with 100mM ammonium acetate (81:19 v/v, pH 6.0) as mobile phase. The isocratic flow rate was maintained as 1.0mL/min at 2200 Psi by keeping column temperature constant at 40℃. The 5µl volume of sample was used for analysis. Vitamins such as B1, B2, B3, B4, B6, B10 and C were identified at 254nm.

Standard Curve: Stock solutions of vitamins as 1mg/2mL were prepared by using 2mL methanol: water

(50:50v/v) as solvents. Various concentrations ranging from 1.0µg/mL to 5.0µg/mL were taken for preparing standard curve. The curve depicted peak area vs concentration of standard was used for linear regression analysis which assisted to determine the analytes.

I. Statistical Analysis

Results represented in the form of mean ± standard deviation and the best fit method used for regression analysis. Regression equation was used to get IC_{50} values i.e. 50% inhibitory concentration.

III. RESULTS AND DISCUSSION

Adequate concentration of sodium hypochlorite used for seed surface sterilization without affecting their growth rate. On tenth day wheat seedlings length was observed as 13.67 cm ± 0.289 (Fig. 1). Harvesting was carried out with sterile knife and juice extracted under hygienic conditions. The 235.36g wheatgrass harvested from 200g wheat grains on tenth day which further used to extract 150mL juice and after lyophilization 6g powder was obtained as data represented in Table 1.

Table 1: Data of wheatgrass harvesting, juice extraction and lyophilization as per tray.

Wheatgrass Extraction/Tray	
Wheat grains	200 g
Wheatgrass	235.36 g
Wheatgrass Juice	150 mL
Wheatgrass Juice Powder (WGJP)	6.0 g
Fresh fibre weight after juice extraction	77.1 g
Dry fibre weight	16.805 g

Lyophilization or freeze drying is a dehydration process used to preserve perishable materials and transport them easily. Lyophilization assisted to increase the viability of heat sensitive components. It maintained the purity of compound by controlling enzymatic and bacterial actions [19]. Whole procedure carried out at low pressure and temperature which is suitable for retaining heat-labile compounds. Sample passed through three main phases freezing, primary and secondary drying to acquire final formulation with lesser moisture content [20].

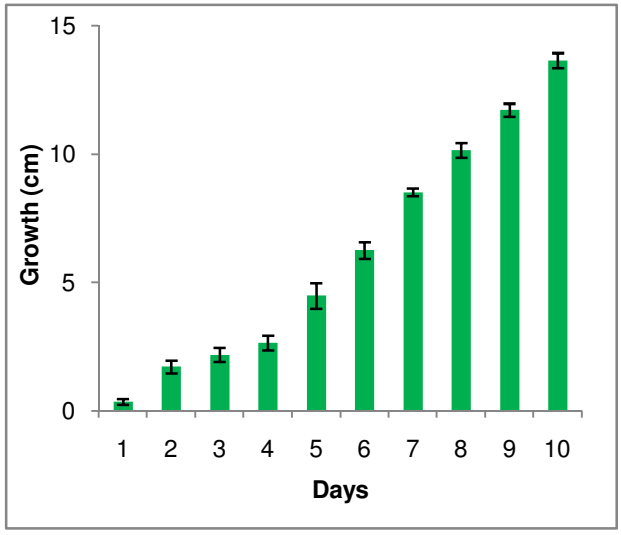

Fig. 1. Wheat seedlings growth measurement. Data represented as mean ± standard deviation.

The lyophilized powder was subjected for pesticide analysis by following GC-MS method in which all the pesticides were detected below detection limit as presented in Table 2.

The chemical composition of lyophilized wheatgrass juice powder was observed as 14 ± 0.283% ash content, 3.5 ± 0.071% moisture content, 3°Bx total soluble solids, 0.25 ± 0.014% acidity and 12 ± 0.707% brix-acid ratio. Moisture content considered as most important property of food samples i.e., related to shelf-life. The moisture content of powdered products within the range of 3–10 % was accepted by food industries on practical basis [21].

Table 2: Pesticide analysis of lyophilized wheatgrass juice powder.

S.No.	Pesticide analysis	Results	S.No.	Pesticide analysis	Results
1.	Alachlor	BDL	22.	Ethion	BDL
2.	Aldrin	BDL	23.	Fenilrothion	BDL
3.	Alpha-Endosulfan	BDL	24.	Fenofos	BDL
4.	Alpha-HCH	BDL	25.	Fenvalerate	BDL
5.	Azinophosmethyl	BDL	26.	Heptachlor	BDL
6.	Beta-Endosulfan	BDL	27.	Heptachlor Endoepoxide	BDL
7.	Beta-HCH	BDL	28.	Heptachlor Exoepoxide	BDL
8.	Brompropylate	BDL	29.	Hexachlorobenzene	BDL
9.	Chlordane	BDL	30.	Lindane	BDL
10.	Chlorfenvinphos	BDL	31.	Malathion	BDL
11.	Chlorpyriphos	BDL	32.	Methidathion	BDL
12.	Chlorpyriphosmethyl	BDL	33.	o-p-DDT	BDL
13.	Counaphos	BDL	34.	Pentachloraniline	BDL
14.	Cypermethrin	BDL	35.	Permethrin	BDL
15.	Delta-HCH	BDL	36.	Phosalone	BDL
16.	Diazinon	BDL	37.	Piperonylbutoxide	BDL
17.	Dichlorous	BDL	38.	Pirimiphos-methyl	BDL
18.	Dieldrin	BDL	39.	p-p-DDD	BDL
19.	Endosulfan Sulphate	BDL	40.	p-p-DDE	BDL
20.	Endrin	BDL	41.	p-p-DDT	BDL
21.	Quintozene	BDL	42.	Propexus	BDL

BDL depict Below Detection Limit

Crude fats, protein and fibres were analyzed by kjeldahl methods as 5.45 ± 0.212, 21.87 ± 1.252 and 1.4 ± 0.085. Spectrophotometrical analysis of carbohydrates, total sugars and reducing sugars was carried out by taking glucose as standard and its values analyzed by using calibration curves of standard (Fig. 2, 3, 4).

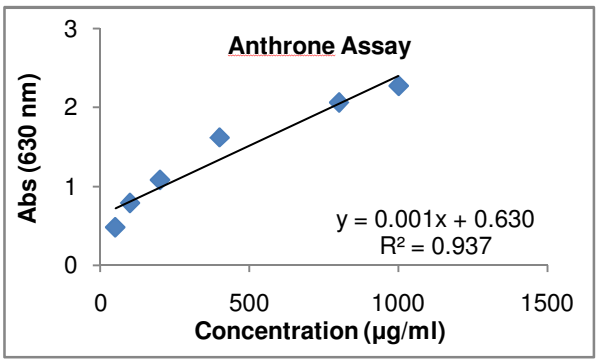

Fig. 2. Calibration curve for carbohydrate analysis.

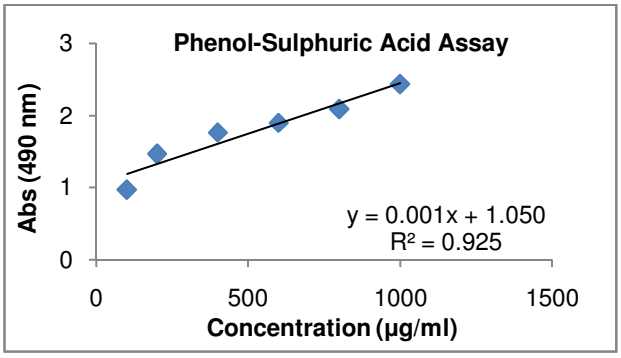

Fig. 3. Calibration curve for total sugar analysis.

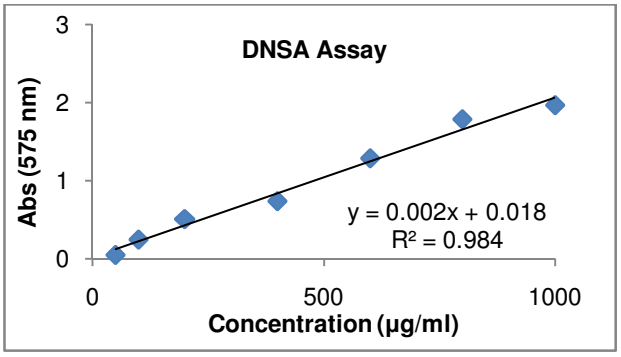

Fig. 4. Calibration curve for reducing sugar analysis.

The carbohydrate content, total sugars and reducing sugars in WGJP was determined as 361mg/g GluE, 17.75 ± 1.06 mg/g GluE and 13 ± 0.707 mg/g GluE. These all chemical constituents that analyzed in wheatgrass juice powder (WGJP) depicted in Table 3. The detection of highest ash content has represented availability of minerals that indicated the existence of necessary elements responsible for chlorophyll biosynthesis. Photosynthetic activity is directly proportional to the amount of chlorophyll content which assisted to accumulate mineral content.

The presence of chlorophyll helped to reverse the toxic effects of oxidation stress and the porphyrin ring in it behaved as anti-mutagen which attacked on carcinogenic materials and have potential for radical scavenging and metal chelation [22].

Table 3: Chemical composition of wheatgrass juice powder.

Nutrients	WGJP
Carbohydrate (GluE mg/g dry wt. of extract)	361± 5.65
Total Sugars (GluE mg/g dry wt. of extract)	17.75 ± 1.06
Reducing Sugar (GluE mg/g dry wt. of extract)	13± 0.707
Brix value	3 ± 0
Acidity (%age)	0.25± 0.014
Brix Acid ratio	12± 0.707
Ash Content (%age)	14± 0.283
Moisture Content (%age)	3.5± 0.071
Crude Fats (%age)	5.45± 0.212
Crude Protein Content (%age)	21.87± 1.252
Crude Fibres (%age)	1.4± 0.085

The WGJP contained 7.46 mg/g total chlorophyll content that carried higher chlorophyll b content i.e., 4.61 mg/g than chlorophyll a (2.85 mg/g) and Xanthophylls + carotenoids determined as 17.31mg/g (Fig. 5). The WGJP extract (80 methanol: 20 distilled water) was subjected to determine radical scavenging potential by following DPPH assay which manifested 50.72 ± 1.87% inhibitory activity with IC_{50} - 980.0µg/mL as compare to standard ascorbic acid with IC_{50} - 28.106µg/mL (Fig. 6). In previous findings 51% inhibition capacity of WGP (wheatgrass powder) was determined in methanolic extract [23]. Elements played important role as structural constituents in amino acids, proteins and act as co-factors for enzyme and phytopigment synthesis [24]. The fourteen elements have been analyzed in WGJP (Fe>Mg>Zn>Mn>K>Ca>Na>Cu>Al>Se>Ag>Cr>Ni>Co) i.e., shown in Fig. 7. The concentration of these elements was not restricted to their availability in seed i.e., altered due to their existence in growth mediums like soil and water. Wheat grass juice powder has higher concentration of Fe, Mg, Zn and Mn i.e., 66.83 ppm, 64.107 ppm, 32.93 ppm and 26.89ppm respectively. The K (Potassium) was observed as 25.541ppm in WGJP followed by Ca (17.238ppm), Na (5.012ppm) and Cu (4.3 ppm). Elements such as Al, Se, Ni, Co, Ag determined in minute quantity. Zn and Mn played important role as free radical scavengers. Mn (Manganese) related with naturally occurring superoxide free radical scavenging enzyme superoxide dismutase as cofactor (Mn-SOD) and Mn-protein in PS-II [24]. Zn assisted in activation of various enzymatic functioning of plants and triggered heavy metal induced protein and lipid oxidation [25]. The potential of wheatgrass to scavenge free radicals and its chlorophyll content positively correlated with existing quantity of Mg, Zn and K. Mg (Magnesium) is prominent constituent of chlorophyll, present as central element in porphyrinring [26].

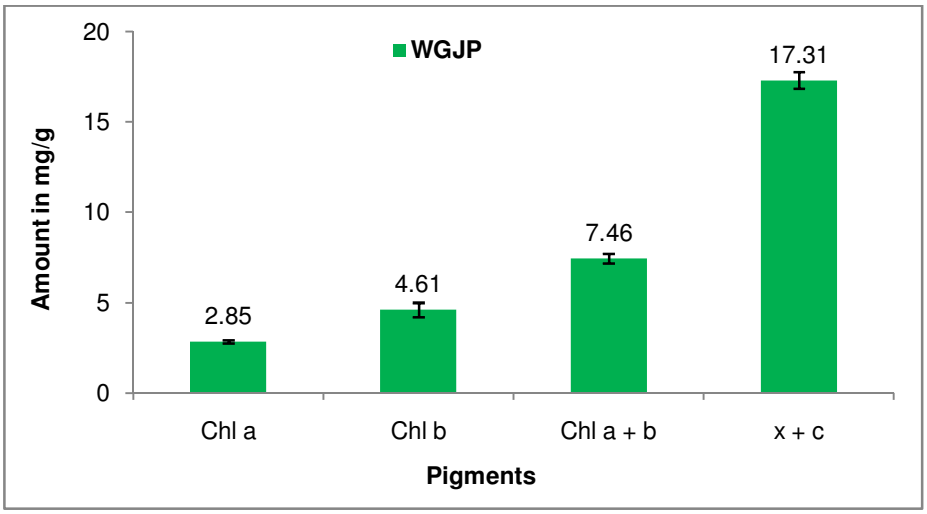

Fig. 5. Chlorophyll estimation of lyophilized wheatgrass juice powder (WGJP). Outcomes represented as mean ± standard deviation.

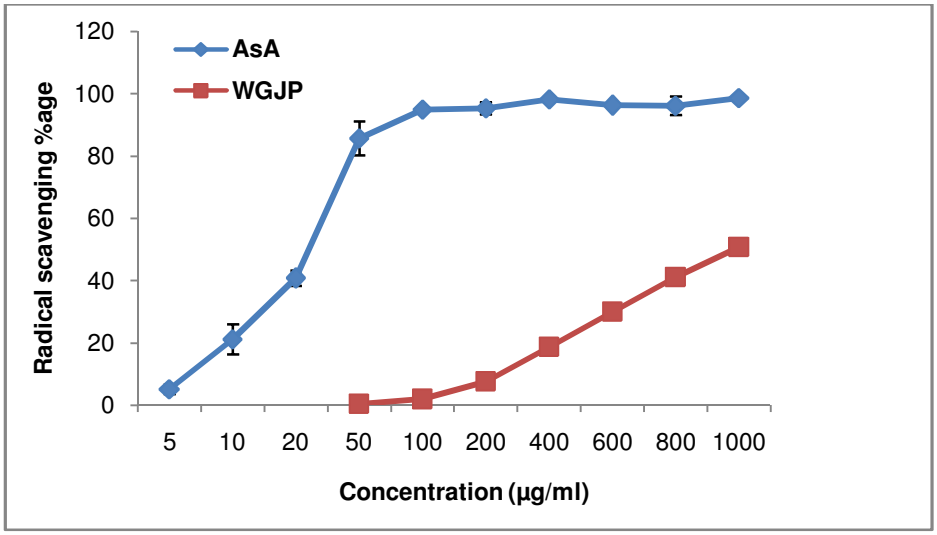

Fig. 6. Radical scavenging capacity of Wheatgrass juice powder extract (80 methanol: 20 distilled water) as compare to standard Ascorbic acid by following DPPH assay. $P < 0.05$.

Fe (Iron) determined as important part of electron transport chain and act as cofactor in various enzymes like peroxidases, cytochromes, xanthine oxidases etc. [27]. Calcium (Ca) participated as cofactor in enzymatic reaction such as oxidation of fatty acids and maintained mineral homeostasis [28]. Cobalt found as chief constituent of vitamin B12 (cobalamin) and induced erythropoietin and metabolizing methionine [29]. Wheatgrass juice powder carried Se in minute quantity i.e., important component of antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase [30]. It has immunomodulatory and proliferation preventive potential that escaped immune cells from oxidative damage [31]. Chromium played significant role in glucose and lipid metabolization [32]. Aluminium (Al), a trace element has beneficial role at lower concentration. It induced root growth, enhanced enzymatic functions and nutrient intake in plants [33].

Water soluble vitamins such as B1, B2, B3, B4, B6, B10 and C were analyzed by exerting HPLC based method in lyophilized wheatgrass juice powder (Fig. 8). The highest amount of vitamin C (9.3mg/100g) was analyzed in WGJP which further followed by vitamin B3 (6.71mg/100g) > vitamin B6 (6.45mg/100g) > vitamin B1 (5.79mg/100g) > vitamin B2 (5.73 mg/100g) > vitamin B10 (5.12mg/100g) and vitamin B4 (4.15 mg/100g). Vitamins played important role as anti-oxidative constituent due to its tremendous potential to scavenge free radicals. It assisted to control hydrogen peroxide liberation within the cells by forming complex with enzymatic antioxidants such as glutathione-ascorbate. Vitamin B-complex also played significant role in free radical scavenging and protect plants from oxidative damage [34].

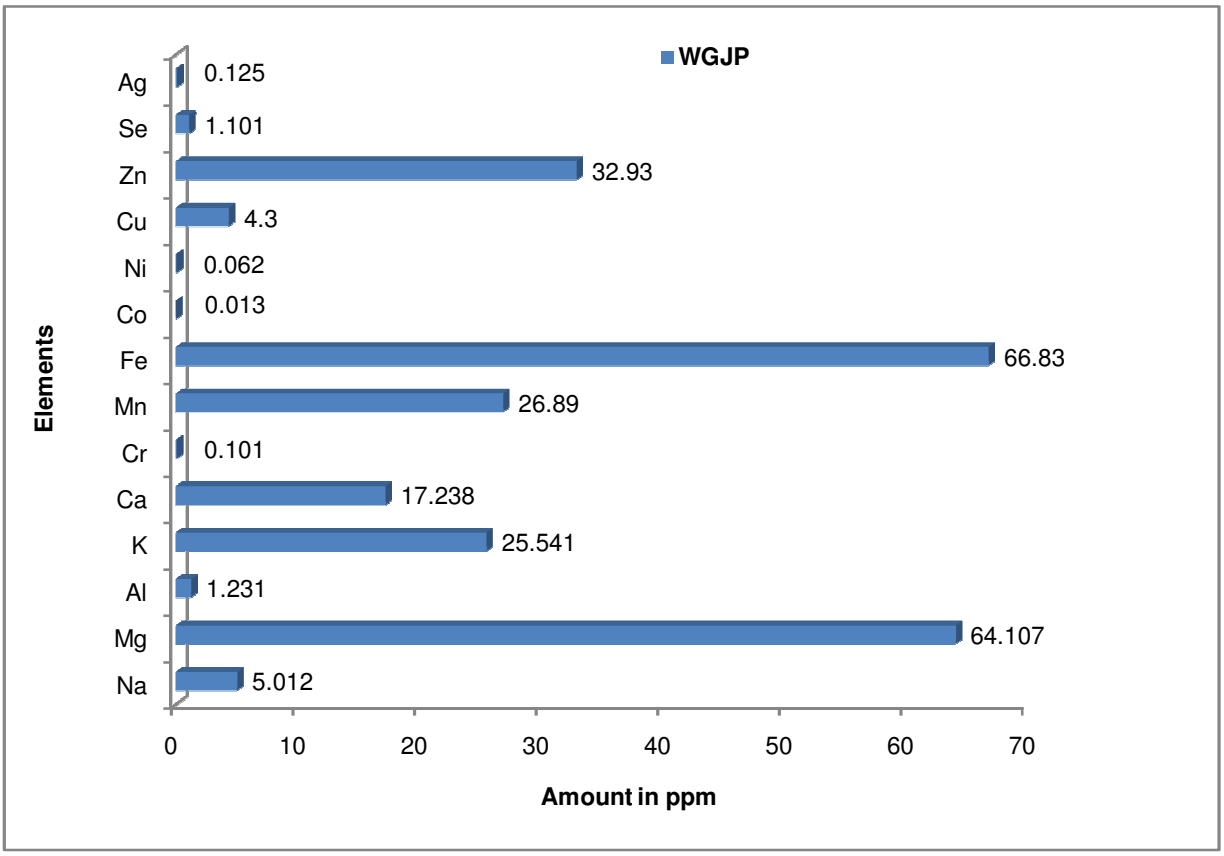

Fig. 7. Elemental analysis of lyophilized wheatgrass juice powder (WGJP) by AAS (Atomic Absorption Spectroscopy). ppm - parts per million.

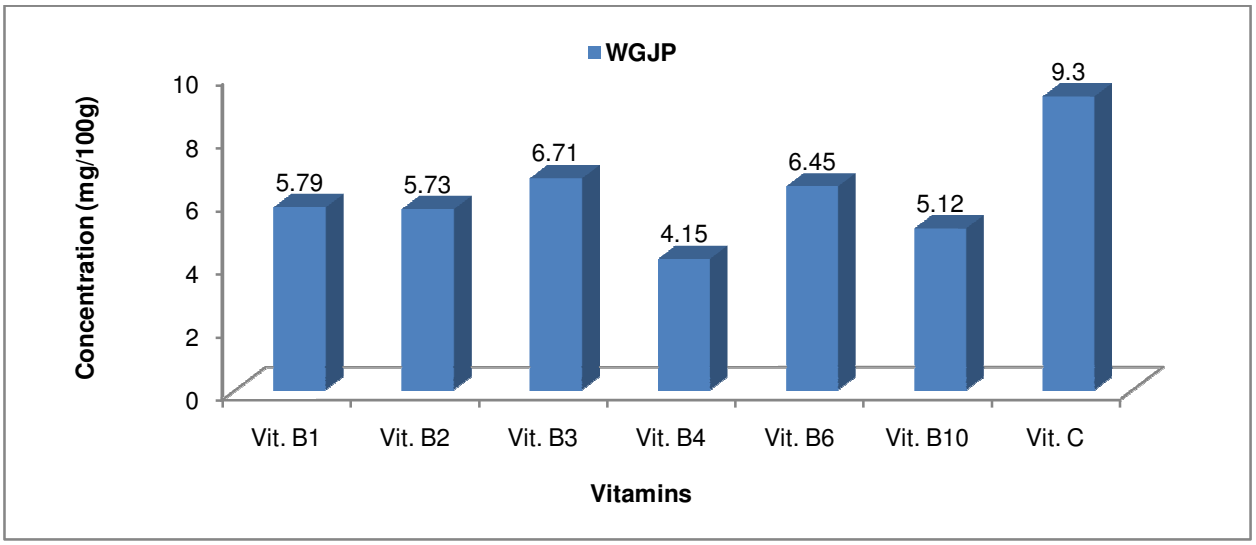

Fig. 8. HPLC based vitamin analysis in lyophilized wheatgrass juice powder (WGJP). (Results for Vitamins are as per Guidelines of FAO/INFOODS Database Version 1.0 & 2.0, Italy).

IV. CONCLUSION

Wheatgrass recognized as life-saving herbal formulation due to its nutritive as well as medicinal aspects. Lyophilization process assisted to protect its heat sensitive constituents that played significant role to block degenerative metabolic disorders. Wheatgrass enriched with chlorophyll content that enhanced its free radical scavenging potency. Lyophilized wheatgrass juice powder carried vitamins, macro and micro elements which accelerated the anti-oxidative mechanisms and controlled the harmful impacts of degenerative diseases such as cancer, arteriosclerosis, diabetes, blood disorders like thalassemia and atopic dermatitis.

V. FUTURE SCOPE

There is need of more research on wheatgrass to explore its therapeutic application in worldwide to make people aware about its multidisciplinary benefits by focusing on isolation of phytoconstituents and to assure its anti-cancerous potential by using multiple cell lines. Wheatgrass products longevity can be elevated by increasing their shelf life. Therapeutically competent herbs can be incorporate to enhance the potentiality of wheatgrass by formulating bizarre of herbal products.

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Conflict of Interest. We declare that we have no conflict of interest.

REFERENCES

[1]. Lai, C. N. (1979). Chlorophyll: The active factor in wheat sprout extract inhibiting the metabolic activation of carcinogens in vitro. *Nutrition and Cancer, 1*, 19–21. doi: 10.1080/01635587909513623.

[2]. Pandey, V., Chura, A., Pandey, H. K., & Nasim, M. (2015). Estimation of ascorbic acid, ß carotene, total chlorophyll, phenolics and antioxidant activity of some European vegetables grown in mid hill conditions of western Himalaya. *Journal on New Biological Reports*, *4*(3), 238–242.

[3]. Arshad, M., Deljou, M. J. N., Haghshenas, M., & Gholizadeh, S. K. (2015). Anti-oxidative capacity and quality of strawberry fruit (Fragaria × ananassa Duch 'Selva') under the balance of minerals nutrition. *Biological Forum – An International Journal*, *7*(2), 91-97. Research Trend.

[4]. Anderson, J. (1986). Fiber and health: an overview. *American Journal of Gastroenterology, 10*, 892-897. PMID: 3020967.

[5]. Bar-Sela, G., Tsalic, M., Fried, G., & Goldberg, H. (2007). Wheat grass juice may improve hematological toxicity related to chemotherapy in breast cancer patients: a pilot study. *Nutrition and cancer*, *58*(1), 43-48.doi: 10.1080/01635580701308083.

[6]. Calzuola, I., Marsili, V., & Gianfranceschi, G. L. (2004). Synthesis of antioxidants in wheat sprouts. *Journal of agricultural and food chemistry*, *52*(16), 5201-5206.doi: 10.1021/jf0307752.

[7]. Nanasombat, S., Thonglong, J., & Jitlakha, J. (2015). Formulation and characterization of novel functional beverages with antioxidant and anti-acetylcholinesterase activities. *Functional Foods in Health and Disease*, *5*(1), 1-16. doi: 10.31989/ffhd.v5i1.162.

[8]. Hamilton, E., Whitney, E., & Sizer, F. (1988). Nutrition: Concepts and Controversies. 4th ed., St. Paul, Minn., West Publishing Co.

[9]. Ben-Arye, E., Goldin, E., Wengrower, D., Stamper, A., Kohn, R., & Berry, E. (2002). Wheat grass juice in the treatment of active distal ulcerative colitis: a randomized double-blind placebo-controlled trial. *Scandinavian journal of gastroenterology*, *37*(4), 444-449. doi: 10.1080/003655202317316088.

[10]. AOAC. (1990). Official methods of analyses of association of analytical chemist.15th eds., Washington DC.

[11]. Ranganna, S. (1986). Handbook of analysis and quality control for fruit and vegetable products.Tata McGraw-Hill Education.

[12]. Gould, W. A. (1978). Quality evaluation of processed tomato juice. *Journal of Agricultural and Food Chemistry*, *26*(5), 1006-1011. doi: 10.1021/jf60219a049.

[13]. Hedge, J. E., & Hofreiter, B. T. (1962). Carbohydrate Chemistry. Whistler RL and Be Miller JN. pp. 17.

[14]. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical chemistry*, *28*(3), 350-356. doi: 10.1021/ac60111a017.

[15]. Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical chemistry*, *31*(3), 426-428. doi: 10.1021/ac60147a030.

[16]. Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant physiology*, *24*(1), 1. PMID: 16654194.

[17]. Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181(4617), 1199-1200. doi: 10.1038/1811199a0.

[18]. Parmar, N., Virdi, A. S., Singh, N., Kaur, A., Bajaj, R., Rana, J. C., Agrawal, L., & Nautiyal, C. S. (2014). Evaluation of physicochemical, textural, mineral and protein characteristics of kidney bean grown at Himalayan region. *Food research international*, *66*, 45-57.doi: 10.1016/j.foodres.2014.08.048.

[19]. Carpenter, J. F., Pikal, M. J., Chang, B. S., & Randolph, T. W. (1997). Rational design of stable lyophilized protein formulations: some practical advice. *Pharmaceutical research*, *14*(8), 969-975.doi: 10.1023/A:101218070.

[20]. Jeff, S. J. (2009). Basic Cycle Development Techniques for Lyophilized Products. *35*, 126-128.

[21]. Li, C., Wang, J., Shi, J., Huang, X., Peng, Q., & Xue, F. (2015). Encapsulation of tomato oleoresin using soy protein isolate-gum aracia conjugates as emulsifier and coating materials. *Food Hydrocolloids*, *45*, 301-308. doi: 10.1016/j.foodhyd. 2014.11.022.

[22]. Endo, Y., Usuki, R., & Kaneda, T. (1985). Antioxidant effects of chlorophyll and pheophytin on the autoxidation of oils in the dark. II. The mechanism of antioxidative action of chlorophyll. *Journal of the American Oil Chemists' Society*, *62*(9), 1387-1390.doi: 10.1007/BF02545965.

[23]. Pasha, I., Huma, N., Chughtai, M. F. J., Jan, S., Ahmad, S., Manzoor, M. S., & Ahmed, F. (2018). Biochemical, nutritional and end use perspectives of wheatgrass as potential dietary supplement. *International Journal of Biochemistry Research & Review*, 1-13. doi: 10.9734/IJBCRR/2018/39539.

[24]. Balashouri, P., & Prameeladevi, Y. (1995). Effect of zinc on germination, growth and pigment content and phytomass of *Vignaradiata* and *Sorghum bicolor*. *Journal of Ecobiology*, *7*, 109-114.

[25]. Halliwell, B., & Gutteridge, J. M. (2015). Free radicals in biology and medicine. Oxford University Press, USA.
[26]. Zago, M. P., Verstraeten, S. V., & Oteiza, P. I. (2000). Zinc in the prevention of Fe_2 initiated lipid and protein oxidation. *Biological research*, *33*(2), 143-150. doi: 10.4067/S0716-97602000000200014.
[27]. Satyanarayana, U., & Chakrapani, U. (2008). Essentials of Biochemistry. 2nd ed., Arunabha Sen Book and Allied (P) Ltd., Kolkata, India, p. 210-227.
[28]. Strain, J. J. S., & Cashman, K. D. (2009). Minerals and trace elements. In Gibney, M. J., Lanham-New, S. A., Cassidy, A., Vorster, H., eds. Introduction to Human Nutrition, ed., 2. The Nutrition Society Textbook Series. Chichester, Wiley-Blackwell, p 188–237.
[29]. Barceloux, D. G. (1999). Cobalt. *Journal of Toxicology: Clinical Toxicology,* 37, 201-206.doi: 10.1081/CLT-100102420.
[30]. Rayman, M. P. (2012). Selenium and human health. *The Lancet*, 379(9822), 1256–1268. doi: 10.1016/S0140-6736(11)61452-9.
[31]. Serwin, A. B., Wasowicz, W., Gromadzinska, J., &Chodynicka, B. (2003). Selenium status in psoriasis and its relations to the duration and severity of the disease. *Nutrition*, *19*(4), 301–304. doi: 10.1016/S0899-9007(02)01081-X.
[32]. Krejpcio, Z. (2001). Essentiality of chromium for human nutrition and health. *Polish Journal of Environmental Studies*, *10*(6), 399–404.
[33]. Bojorquez-Quintal, E., Escalante-Magana, C., Echevarria-Machado, I., & Martinez-Estevez, M. (2017). Aluminum, a Friend or Foe of Higher Plants in Acid Soils. *Frontiers in Plant Science,* 8, 1767. doi: 10.3389/fpls.2017.01767.
[34]. Asensi-Fabado, M. A., & Munne-Bosch, S. (2010). Vitamins in plants: occurrence, biosynthesis and antioxidant function. *Trends in Plant Science, 15*(10), 582–592. doi: 10.1016/j.tplants.2010.07.003.

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