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RESEARCH ARTICLE

ADDRESSING FOOD SAFETY CONCERN AND PROMOTING BETTER HUMAN HEALTH THROUGH HYDROPONICALLY GROWN NUTRITIONAL POWERHOUSE.

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Abstract

Fertilizers, agrochemicals and pesticides, when used in higher quantities than needed, become contaminants to food, feed and environment. This has prompted international organizations to establish standards for the safe production of fresh crops that can be safely consumed. To provide a wholesome nutrition for a healthy and rejuvenating body, green food could be very useful in providing nutrients like vitamins, minerals, proteins, enzymes and antioxidants which are researched for numerous health benefits. Wheat grass is a nutritional powerhouse, rich in chlorophyll, antioxidant vitamins, minerals and other nutrients that support optimal health contains significant amount of iron, phosphorus, magnesium, manganese, copper & zinc. In house hydroponically grown wheat grass, without any use of fertilizer or pesticide, taken up for juice preparation under hygienic conditions was analyzed for microbiological safety and various nutritional parameters.

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Introduction:-

Food Safety is increasingly viewed as an essential public health issue in the developed and developing countries. The rapidly changing and globalizing food economy and the concerns and commitments of a wide range of stakeholders about food production and security, food safety and quality, and the environmental sustainability of agriculture have prompted international organizations to establish standards for the safe production of fresh crops that can be safely consumed. Fertilizers, agrochemicals and pesticides, when used in higher quantities than needed, become contaminants to food, feed and environment. However, when used properly, they will improve crop yield and quality. FAO (Food and agriculture organization) has established Good Agricultural Practices (GAPs) and defined this concept as: "Applying available knowledge to addressing environmental, economic and social sustainability for on-farm production and post-production processes that result in safe and healthy food and non-food agricultural products."

To provide a wholesome nutrition for a healthy and rejuvenating body, green food could be very useful in providing nutrients like vitamins, minerals, proteins, enzymes and antioxidants which are researched for numerous health benefits (4). Wheat grass the mature shoot of *Triticum aestivum* Linn. belonging to the family Gramineae is a nutritional powerhouse, rich in chlorophyll, antioxidant vitamins, minerals and other nutrients that support optimal

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health contains significant amount of iron, phosphorus, magnesium, manganese, copper & zinc. It is a rich source of tocopherols with high vitamin E potency and biotin, which is a crystalline form of the vitamin B-complex, essential for the activity of many enzyme systems (2). The three most important effects of wheat grass on the human body are: blood purification, liver detoxification and colon cleansing.

Studies have been carried out to assess wheat grass juice's beneficial effect in alleviating specific health issues like diabetes and its complications. It was observed that it reverses hyperglycemia, has an efficacy on hemoglobin level, RBCs and platelets count indicating therapeutic usefulness of wheatgrass in some types of anemia. In addition, it produces anti-inflammatory and a potent immunomodulatory effects, therefore it could be used as an immune booster in diabetic subjects suffering from low immunity (3).

To address the issues related to food safety and make sincere efforts for wider acceptability of wheat grass juice and its maximum benefits to human health, Ayurved Research Foundation, village Chidana, district Sonapat, Haryana took the initiative and grew the crop hydroponically at their premises under controlled conditions, without any use of fertilizer or pesticide, with optimal supply of nutrients. Its juice was prepared in house under hygienic conditions and analyzed for microbiological safety and various nutritional parameters.



Fig. 1

Fig. 2

Fig. 3

Fig. 1:-Hydroponic machine at Ayurved Research Foundation; **Fig. 2- Wheat grass grown in Hydroponic machine from 0 day to harvesting ; Fig. 2- Wheat grass ready to harvest**

Material and Method:-

750 g of good quality wheat seeds were soaked in water for 24 hours and put in tray in hydroponic machine maintained at temperature $18 \pm 1^\circ\text{C}$ and RH $65 \pm 5\%$. The seeds were irrigated with appropriate nutrient mixture for 7 days and the leaf portion was harvested, washed properly and subjected for juice preparation using a blender and press filtration.

Apparatus:-

Kjeldahl assembly was used for the estimation of proteins, UV absorbance was taken on SHIMADZU UV 1700 UV VIS spectrophotometer, sodium was evaluated on Harrison's flame photometer.

Reagents &Material:-

Chemicals and reagents used were of analytical reagent grade. Petroleum ether, chloroform, HPLC water, ammonia solution, sodium hydroxide, potassium hydroxide & potassium thiocyanate were from RANKEM. Sulphuric acid, citric acid & hydrochloric acid were of SD fine chemicals. Ferrous sulphate was from HIMEDIA, potassium persulphate was from JT Baker, sodium diethyldithiocarbamate was from Sigma Aldrich. Other chemicals used were of AR grade and procured from authentic sources.

Methodology for the estimation of Fat:-

Defat the sample with petroleum ether ($60-80^\circ\text{C}$) Transfer the filtrate to a tared petridish portion wise and evaporate to dryness on a boiling water bath. Cool the petridish in desiccator and weigh. The extractive value is calculated as a percentage.

Methodology for the estimation of Crude fibre:-

Defat the sample with petroleum ether ($60-80^\circ\text{C}$), reflux the marc sequentially in 0.255 N H_2SO_4 and 0.313 M NaOH, wash with 1.25 % H_2SO_4 , water and ethyl alcohol. Dry and ignite the residue in silica crucible at 600°C for 30 minutes. Cool the crucible in a dessicator and weigh for a constant weight and carry out the calculations.

Methodology for the estimation of Protein:-

Digest the sample with potassium sulphate and copper sulphate in 9:1 ratio in a digestion tube using concentrated H_2SO_4 at 400 °C for 35 minutes. Cool and add 100 ml of distilled water, transfer in a RBF and add 40% NaOH, colour of the solution becomes blue. Add boiling chips and attach the RBF to Kjeldahl distillation apparatus, outlet of the apparatus is attached with conical flask having boric acid solution with indicator. The distillation outlet tube is dipped in the boric acid solution. Distill the RBF mixture until the volume of conical flask containing boric becomes more than 150 ml. Titrate the distillate with 0.1N hydrochloric acid solution.

Methodology for the estimation of Ash:-

Ignite a known amount of sample placed in silica crucible in a muffle furnace at 750 °C for 5 hours and cool. Weigh the crucible till constant weight and calculate the % ash.

Methodology for the estimation of Carbohydrate:-

Digest the sample with 2.5 N HCl. Develop the color using anthrone reagent and take absorbance at 630 nm using glucose as standard. Calculate the result using linear regression curve plot.

Methodology for the estimation of Calcium:-

Digest the ash of accurately weighed sample with conc. HCl for 10 minutes and prepare the sample in HPLC grade water. Carry out the complexometric titration with EDTA using hydroxy naphthol blue indicator with color point pink to blue.

Methodology for the estimation of Phosphorus:-

Digest the sample with sulphuric acid. Cool and add nitric acid, boil till colorless solution is obtained. Develop the color with molybdovanadate reagent and take UV absorbance. Calculate the result using linear regression curve plot.

Methodology for the estimation of Sodium:-

Prepare the sample by dissolving it in HPLC grade water, filter the solution before subjecting to Flame photometer. Use analytical grade sodium chloride as standard. Calculate the result using linear regression curve plot.

Methodology for the estimation of calorific value by bomb calorimeter:-

Weigh accurately about 1.0 g of sample pellet in crucible. Place a nichrome wire across the electrodes and tie a thread touching with sample pellet. Charge the bomb with oxygen gas. Place the bomb in calorimeter vessel and make all the connections. Pour the measured quantity of water into the calorimeter and start the mixer. After 10 minutes of mixing, adjust the digital temperature meter to zero. Press the ignition button. Wait till the temperature raise to a constant value, record it and calculate the calorific value.

Methodology for estimation of total antioxidant activity:-

The antioxidant activity is calculated on the basis of the scavenging activity of the stable 2, 2- diphenyl-2-picrylhydrazyl (DPPH) free radical according to the method described by Brand-Williams et.al. (1) with slight modification. The different concentrations of the juice were prepared in ethanol. The test tubes are incubated for 30 min at room temperature and the absorbance is measured at 517nm. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. Ascorbic acid is used as a standard and the concentrations prepared are same as of the test solutions. The difference in absorbance between the test and the control (DPPH in ethanol) is calculated and expressed as % scavenging of DPPH radical. The free radical scavenger activity is calculated in terms of Ascorbic acid equivalent antioxidant capacity (AEAC).

Methodology for the estimation of TPC (IS 5402:2012) : Weigh $10.0 \pm 1.0g$ of sample and transfer into 250 ml conical flask and add sterile 90 ml maximum recovery diluent , mix and prepare serial dilutions. Inoculate in duplicate 1 ml of inoculum from each dilution in plate count agar media & incubate at 30°C for 72 hrs in invert position, count the colonies using colony counter.

Methodology for the estimation of Yeast & Mould (IS 5403:1999):-

Weigh $10.0 \pm 1.0g$ of sample and transfer into 250 ml conical flask, add sterile 90 ml maximum recovery diluent , mix and prepare serial dilutions. Inoculate in duplicate 1 ml of inoculum from each dilution in chloramphenicol yeast extract agar & incubate at 25°C for 5 days in upright position, count the colonies using colony counter.

Methodology for the estimation of E Coli [IS 5887(part-1): 1976]:-

Weigh 25.0 ± 1.0 g of sample and transfer into 250 ml conical flask, add sterile 200 ml peptone water diluent, mix and inoculate 10 ml of initial suspension in to 10.0 ml of MCB (MacConkey Broth), streak on MCA (MacConkey Agar) and EMBA (Eosin Methylene Blue Agar), incubate at 37°C for 24 hrs. in inverted position. Observe the gas production in MCB, if it is there then typical E. coli on MCA are observed as pink colonies & on EMBA as dark centred colonies with or without green metallic sheen. If no gas production is observed in MCB then no typical E. coli on MCA & EMBA shall be observed.

Methodology for the estimation of Salmonella [IS 5887 (part-3): 1999]:-

Weigh 25.0 ± 1.0 g of sample and transfer into 250 ml conical flask, add sterile 225ml buffered peptone water, mix and incubate at 37°C for 16 - 20 hrs. Transfer 0.1 ml pre-enrichment mixture to 10 ml of RV(Rappaport-Vassiliadis) medium and another 10 ml mixture to 100 ml of SCB (Selenite Cystine) medium. Incubate RV at 42°C for 18 - 24 hrs & SCB: 37°C for 24 - 48 hrs. Inoculate streak loopful of mixture from RV and SCB onto BGA (Brilliant green Agar) and XLD (Xylose Lysine Desoxycholate) agar & incubate at 37°C for 24 - 48 hrs. Observe colonies of Salmonella on BGA as pink / red colonies and on XLD as pink colonies with or without black centers.

Result and Discussion:-

Wheatgrass is an inexpensive and efficient source to provide all the required nutrients and medicinal benefits for a healthy and rejuvenating body. Its fresh juice is very good for human consumption as it provides chlorophyll, amino acid, vitamins, enzymes, significant amount of mineral nutrients like iron, phosphorus, magnesium, manganese, copper & zinc and is rich source of tocopherols with high vitamin E potency (2). Wheat grass juice prepared out of hydroponically grown wheat grass without using any pesticide or fertilizer at Ayurved Research Foundation, village Chidana, district Sonapat, Haryana was analyzed for various nutritional parameters and antioxidant activity (Table. 1) and for bio burden (Table. 2) at ARF's DSIR recognized state of the art R&D Centre.

Table 1:- Nutritional composition of wheat grass juice.

Parameters	Result [#]
Antioxidant activity, %	91.87
Ash content, % (w/w)	0.40
Crude protein, % (w/w)	3.31
Crude fiber, % (w/w)	0.48
Carbohydrate, % (w/w)	10.48
Calcium, % (w/w)	0.70
Ether soluble extractive, % (w/w)	4.14
Metabolizable energy, Kcal	484.70
Potassium, % (w/w)	ND
Phosphorus, % (w/w)	0.0063
Sodium, % (w/w)	0.00075
pH	6.60

Mean of 6 samples

Table 2:- Pathogenic load of wheat grass juice.

Name of parameter	Result
Total plate count cfu/g	<10 cfu/g
Yeast and mould count cfu/g	<10 cfu/g
E. coli Detection/25gm	Absent
Salmonella Detection/25gm	Absent

Conclusion:-

The hydroponic technique for growing the green food/ wheat grass which is absolutely free of pesticide/ agrochemicals residues is a key step towards addressing the food safety concerns and providing the wholesome nutrition. The juice prepared out of it is a good source of proteins, strong antioxidant property apart from other nutrients which are essential for a healthy and rejuvenating body. The bio burden load was also found to be within

specified limits as per standard guidelines. It is suggested that wheatgrass juice should be a part of daily dietary intake in order to obtain its maximum benefits.

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