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PHYTOCHEMICAL SCREENING AND ESTIMATION OF NUTRITIONAL CONTENT OF WHEATGRASS POWDER AND WHEATGRASS JUICE

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ABSTRACT

Wheatgrass is rich in many minerals, vitamins, amino acids, proteins, carbohydrates, chlorophyll, enzymes that are useful for our body. Now-a-days, many formulations of wheatgrass are available in market, but no proper study is available to compare the effectiveness between wheatgrass powder and wheatgrass juice. Present work of estimate the phytochemical screening and nutritional content present in both wheatgrass powder and fresh wheatgrass juice, based on chemical investigation and spectroscopy which is simple, inexpensive and less time-consuming method. This method is properly validated using standard chemicals. In our project, the contents of Chlorophyll and Vitamin C in wheatgrass powder and fresh wheatgrass juice, were determined. Along with estimation of phytochemical screening of active constituents which are extracted in aqueous, chloroform and n-hexane solvents.

KEYWORDS: Wheatgrass (Triticum aestivum) powder and fresh wheatgrass juice, Vitamin C, Chlorophyll, Aqueous, Chloroform, n-hexane.

MATERIALS AND METHODS

1. Procedure for growing wheatgrass

a. Germination of wheat grains

Superior good quality whole wheat was procured, and cleaned properly. The wheat grains were soaked in cold water for 12 hours. After 12 hours of soaking the water was strained and

the soaked grains were tied in wet woven cotton cloth and hung for a period of 12 hours. Water was sprinkled over the cotton cloth at least thrice during germination period.[13]

b. Cultivation of wheat grass

- After 12 hours of germination, the soaked wheat-grain were spread on the surface of the soil filled in plastic trays. A thin layer of soil was sprinkled on the wheat grains and then tray was covered with a newspaper to provide darkness, which helps sprouting.[13]
- The tray was kept in a covered balcony. Next day, the tray was uncovered to spray on some water and was covered again with the newspaper, sprouting took place, after which the tray was left uncovered and watered everyday for 8 days.
- On 9th day the wheatgrass was harvested by cutting it with a clean pair of scissors about 1/2" above the surface of the soil.

2. Sample preparation

a. Preparation of Triticum aestivum (wheat grass) powder and juice[14,15,16]

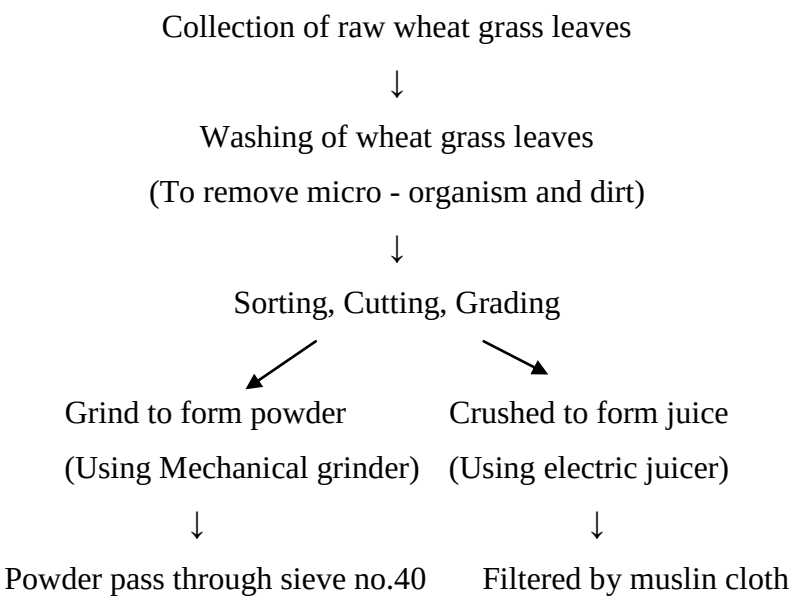


Figure 1: Preparation of wheat grass powder and juice.

B. Preparation of the extracts

Maceration technique was used for the extraction. 10g powder of T. aestivum (grass) was suspended in 100ml of hexane followed by chloroform, methanol and distilled water using 250ml conical flask and kept on orbital shaker for 48 h at 37°C. After 48h, the supernatant was filtered through What man filter paper no.1 and evaporated to dryness at room temperature. The viscous material was stored in sterile, air-tight container. The residue was

dried and further used for successive extraction. In successive extraction, crushed wheatgrass was exhausted by adding small quantities of petroleum ether, benzene several times followed by filtration, every time in a successive manner. This process was repeated sequentially, with chloroform, n-hexane and finally with water.

3. Preliminary pharmacognostic studies

a. Macroscopic studies

The fresh juice and powder are subjected to macroscopic studies which comprised of organoleptic characters of the drugs viz. color, odour, appearance, taste (table 1).[1,4]

b. Physicochemical studies

Physicochemical studies include ash value and extractive value to determine the quality and purity of the powder of plant of Triticum aestivum.[14]

c. Microscopic studies[1,4,14]

Microscopic studies of transverse sections, surface preparations and powder studies of Wheatgrass are conducted using high-resolution microscope.

4. Preliminary phytochemical studies[6,9,14,15,16,17,18,19,20]

Powdered material of T. aestivum grass (50g) was crushed and extracted with 500ml of hexane, chloroform, and water using a Soxhlet apparatus. Each extract was suspended in proper solvent and to identify the phytochemical constituents present in T. aestivum L. grass extracts, a preliminary screening was carried by the application of various testing methods of Draggandorff's test, Hager's test, Wagner's test and Mayer's test, Liebermann-Bur chard test, Foam formation test, Lead acetate test, Molisch's and Felhing's test and Ferric Chloride test for determining the presence of alkaloids, terpenes, steroids, saponins, flavonoids, polysaccharides and tannins, respectively.

5. Determination of Chlorophyll[22,23]

By Colorimetric method:

Material **:** Acetone,
Calcium carbonate,
Wheatgrass sample.

Wavelength **:** 0, 420, 440, 490, 520, 540, 570, 600 & 720 in 1 cm cells.

1. One gram dry wheatgrass powder/ Wheatgrass juice was ground in a glass mortar.
2. Ten ml of acetone was added and the tissues were macerated.
3. A pinch of calcium carbonate was added to this mixture to prevent the degradation of chlorophylls to pheophytin.
4. The mixture was decanted, The liquid was transferred to a 50 ml volumetric flask.
5. The residue in the mortar was mashed further with 5 ml of acetone and themixture was filtered.
6. The residue remaining in the funnel was washed with 2 to 5 ml quantities of acetones till the washings were colourless.
7. The colored extracts were combined.
8. Acetone was added to the flask to make the volume upto the mark (50 ml).
9. The extract was shaken well.
10. One ml of the chlorophyll extract was carefully transferred to a volumetric flask. The liquid was diluted with acetone to 25 ml mark, then titration will be carried out.
11. The measuring cylinder was shaken well and the absorbance was measured in a colorimeter at wavelength at 0, 420. 440, 490, 520, 540, 570, 600 & 720 in 1 cm cells,
12. The Wavelength versus absorbance was plotted on a graph paper.

6. Determination of VitaminC[24,25]

By HPLC method:

a. Analysis by liquid chromatography

Analytical Column **:** Analytical column RP C18 or RP C8 (250 x 4,6 mm, 5mm; 125 ×4,6 mm, 5µm;) with precolumn

Lambda Max **:** 240 nm

Mobile Phase **:** Methanol: water (5:95, *v/v*) pH= 3 (H3PO4).

Flow time **:** 20 min (Flow rate-1 ml/min)

Temperature **:** Laboratory temperature

a. Standard Solution

100 mg sample was dissolved in 100 ml solvent (water) and dilution was made upto 10 µg/ml. Standard was scanned at 240 nm.

b. Sample preparation (Powder and Juice)

100 gm sample / juice was extracted in 100 ml benzene and 100 ml water. Benzene part was drained out. Water part was used for Vitamin C determination by HPLC.

c. Identification and quantification

The identification of the analyte is performed by the comparison of its retention time in analysed sample with the retention time of the calibration standard. Quantitative analysis is performed using the external standard method by the calculating of the concentration in analytical sample from the calibration curve equation.

RESULTS AND DISCUSSION

Triticum aestivum L. Belonging to family Poaceae is a green commonly found herb in India. I decided to work on this plant to find out and compare its nutritional contents of Chlorophyll and Vitamin C in wheatgrass juice and powder with their usefulness to human being. The present works include screening of pharmacognostics evaluation along with its preliminary phytochemical evaluation. Extraction using different organic solvents is avoided considering solubility of chlorophyll.

The study was divided in two parts:-

1. Pharmacognostic studies,
2. Phytochemical studies.

1. Preliminary pharmacognostic studies

a. Macroscopic studies

i) Physical Characteristics

Table1. Physical Characteristics of wheatgrass juice& powder.

Physical Constants	Triticum aestivumL. Juice	Triticum aestivumL. Powder
Macroscopic Characteristics		
Nature	Grass	Grass
Colour	Bright green/ Dark green	Dark green
Odour	Characteristic	Faintly Characteristic
Taste	Acrid	Slightly sweet

ii) Ash values

Table 2: Ash values of Wheatgrass grass.

Sr. NO.	Ash Value	POWDER (% W/W)	JUICE (% W/W)
1	Total Ash	15.1	15.2
2	Acid insoluble ash	5.3	5.2
3	Acid soluble ash	4.9	4.9
4	Water insoluble ash	12.5	12.4
5	Water soluble ash	3.1	3.0

iii) Extractive values

Table 3: Extractive values of Wheatgrass grass in above solvents.

Sr. NO.	Physicochemical parameters	POWDER (% W/W)	JUICE (% W/W)
1	Water soluble extractive value	20	19.9
2	Methanol soluble extractive value	25	24.8
3	Benzene soluble extractive value	15	15.2
4	Choloroform soluble extractive value	5	5.1

b. Microscopic studies

Microscopic studies of transverse sections and powder studies of wheatgrass. The structure of wheatgrass leaf showed elaborate epidermis with characteristic stomata and trichomes, green assimilating parenchyma, conducting vascular bundles and longitudinal strands of fibrous stereome or supporting tissue.

i) Transverse section of Triticum leaf

1. On the upper surface of the leaf there was a series of longitudinal ridges or ribs, the lower surface being almost flat.
2. The epidermal cells covering the ridges differed in form and arrangement from those over the furrows and along the edge of the leaf.
3. The trichomes or hairs were always Unicellular. It had more number of trichomes, mostly in lower epidermis. On the leaves of T.aestivum, ample numbers of hairs were present. These were usually more on the upper epidermis than the lower epidermis.
4. Each stoma on the leaf consisted of four cells, the two guard cells being narrow, The ratio of the number of stomata on the upper and lower epidermis respectively was about 10:7.
5. The parenchymatous cells of outer bundle sheath were larger and alsomore in number. The parenchyma of the leaf consists chiefly of thin-walled assimilating tissue, containing lenticular chloroplasts 4.5-6 cm in diameter.
6. The cells of the chlorophyll-containing tissue in the central part of the leaf were much more irregular in shape and are loosely packed, with large intracellular spaces between them.
7. All vascular bundles were collateral, with the xylem towards the upper surface of the leaf and the phloem bellow. In the xylem there were one or two vessels. Outer sheath of vascular bundle was complete and was composed of elongated thick-walled cells; the outer or ‘parenchyma sheath’ was more conspicuous and consisted of thin-walled cells, almost circular in transverse section.

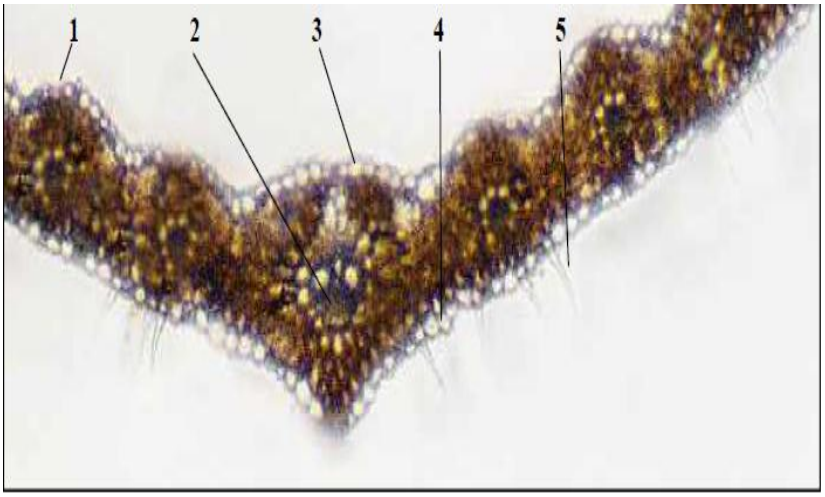


Figure 2: Transverse section of Triticum aestivum.

1. Ridge
2. Vascular Bundle
3. Outer epidermis
4. Inner epidermis
5. Trichome

ii). Powder characteristices of Triticum aestivum (wheatgrass)

1. Epidermal cells in surface view were elongated and rectangular having few numbers of stomata.
2. Trichomes were simple, uniseriate, unicellular and long with pointed end and swollen bases. Smaller ones were hook-shaped with broad base while longer trichomes were more in number than smaller ones.
3. Fibers were scattered here and there, found as single or in groups. They were thin-walled and lignified.
4. Vessels were single or together in groups of 2-3, pitted, reticulated and annular type. Pitted vessels were more in number**.**

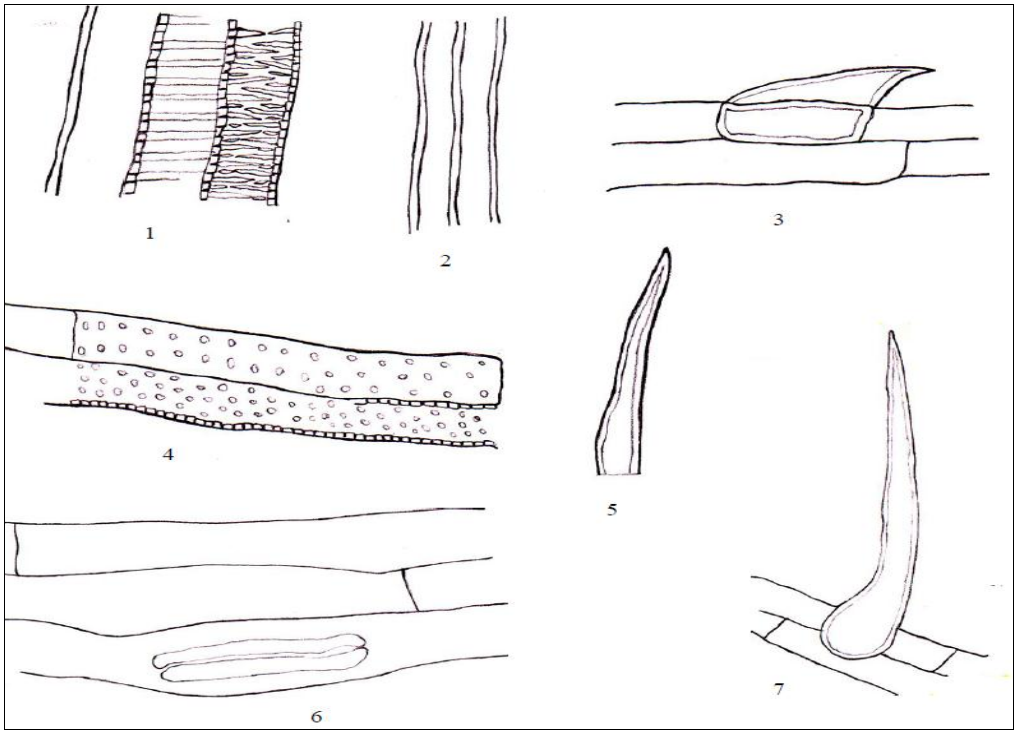


Figure 3: Characteristics of T. aesticum powder.

1. Reticulated vessels
2. Group of fibers
3. Hook-shaped trichome
4. Pitted vessels with pitted parenchyma
5. Broken trichome
6. Epidermise in surface view with stomata
7. Uniseriate, unicellular simple trichome

2. Preliminary phytochemical studies.

Table 4: Results of Phytochemical Investigation of Wheatgrass Powder & Juice.

Name of the Test	Wheatgrass powder			Wheatgrass juice		
	Aqueous	n-hexane	Chloroform	Aqueous	n-hexane	Chloroform
1. **Test for carbohydrates**						
a) Molisch's test	+	-	-	+	+	-
b) Fehling's test	+	-	-	+	+	-
c) Benedict's test	+	-	-	+	+	-
2. **Test for proteins**						
a) Biuret test	+	-	-	+	-	-
b) Xantho protein test	+	-	-	+	-	-
	+	-	-	+	-	-

c) Millons test						
3. **Test for Amino acids** a) Ninhydrin test	+ +	- -	- -	+ +	- -	- -
4. **Test for Alkaloids** a) Dragendroff's test b) Mayer's test c) Hager's test **d)** Wagner's test	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
5. **Test for steroids** a) Salkowski test	-	+	-	-	-	+
6. **Test for phenolics& tannins** a) Ferric chloride test b) Lead acetate test **c)** Dil. HNO3 test	- - -	- - -	+ + +	+ + +	- - -	+ + +
7. **Tests fixed oils and fats** a) Stain test	-	-	-	-	-	-
8. **Test for glycosides** a) Keller-Killiani Test	-	+	+	-	-	+
9. **Test for saponins** a) Haemolytic test **b)** Foam test	+ +	- -	+ +	+ +	- -	- -
10. **Test for triterpenoids** a**)** Salkowski test	-	-	-	-	-	-

(+) = Positive, (-) = Negative

Water soluble extractive value was found to be greater than chloroform and n-hexane soluble extractive value in the experiment. The reason behind that is chlorophyll content of wheatgrass is which is about 70%. Chlorophyll is water soluble.

1. Aqueous extract shown positive test for carbohydrates in juice and powder it may be due to presence of sucrose, glucose and fructose etc while n-hexane extract of shown positive result for carbohydrates it may be due to presence insoluble fiber.
2. Aqueous extract of juice and powder given positive test protein and Aqueous extract of juice and powder given positive test amino acid.

3. Some alkaloid are found in salt form in nature which makes them water soluble and some alkaloids are also lipohilic which makes them water insoluble.
4. As steroids are lipid soluble only n-hexane extract has given positive test.
5. Extra force applied for making juice leaches water soluble tannin which occur in aqueous extract which make it black and will smell bad after course of time just what happens in some river due to which they get blacken. Keeping juice for long time same result are shown by juice that it gets black. So in market always powder are recommended.
6. All extracts shown negative test for fixed oil and fats.
7. Aqueous extract and chloroform extract of juice and powder shown positive test while n-hexane extract powder shown positive test for powder only. During drying sugar-rich glycosides can lose one or more sugar residue due to hydrolysis by glycosidase and due which aglycone part which is lipophilic gets separated from glycone part. Hence the test in n-hexane is positive of powder.
8. Aqueous and chloroform extract shown positive result for saponins.

Phytochemical tests suggested that wheatgrass contains phenolic compounds, flavonoids, proteins and amino acids in water extracts, whereas these were absent in n-hexane and chloroform extract. Alkaloids and cardiac glycosides were not detected in our chemical tests.

3. **Determination of Chlorophyll:**

By Colorimetric method

Nutritional analysis

1. Chlorophyll in Wheatgrass powder.

Concentration : 114 mg/100g

Chlorophyll content in wheatgrass powder was found to be 114 mg/100g.

2. Chlorophyll in Wheatgrass juice

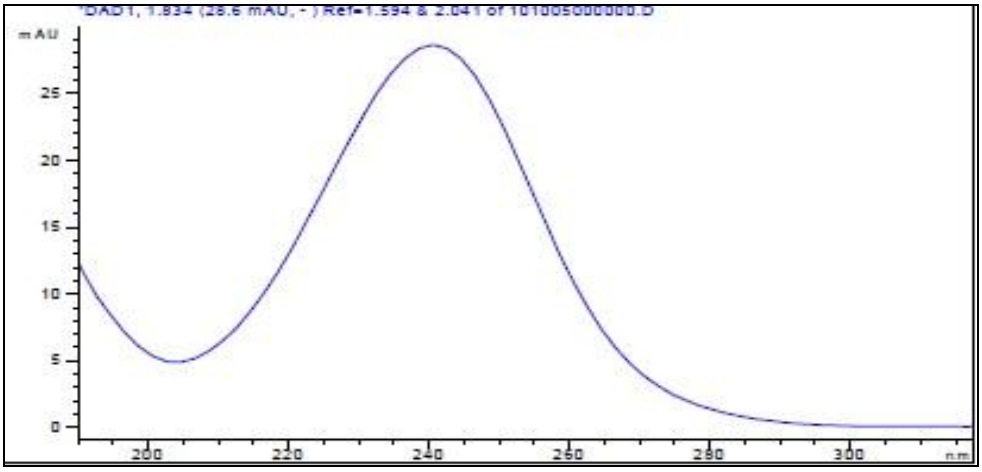
Concentration : 102 mg/100g

Chlorophyll content in wheatgrass powder was found to be102 mg/100g.

From the above observation it is state that, the Chlorophyll content in both Wheatgrass powder and Wheatgrass juice was found to be 114 mg/100g and 102 mg/100g respectively, i. e. powder form contain more Chlorophyll contents as compare to juice form.

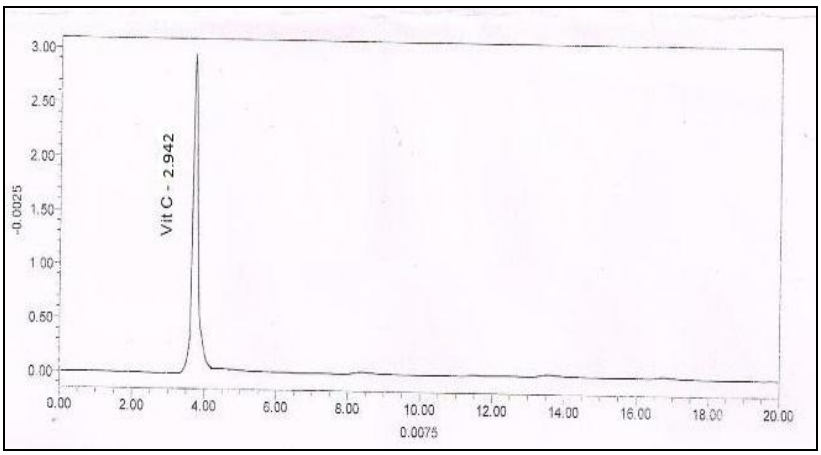
4. Determination of Vitamin C by HPLC method

i) UV spectra of Vitamin C



Graph 1: UV Spectra of Vitamin C.

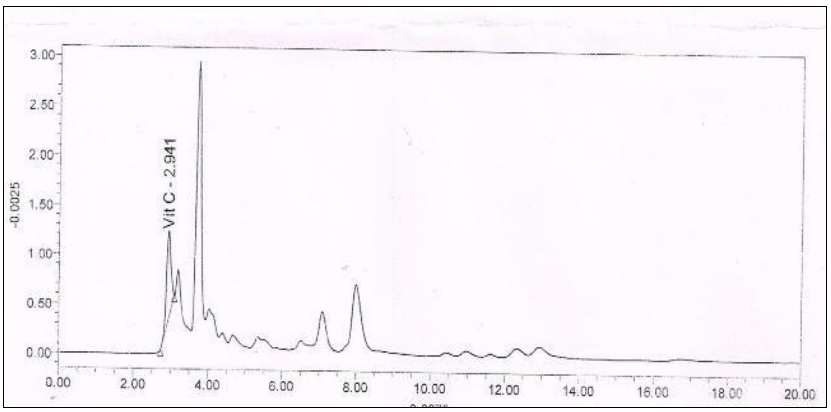
ii) Calibration curve for Vitamin C



Graph 2: Calibration curve for Vitamin C.

Nutritional analysis

1. Vitamin C in Wheatgrass Powder



Graph 3: Calibration curve for Vitamin C in Wheatgrass powder.

λ –max : 240 nm

Retention time : 2.941

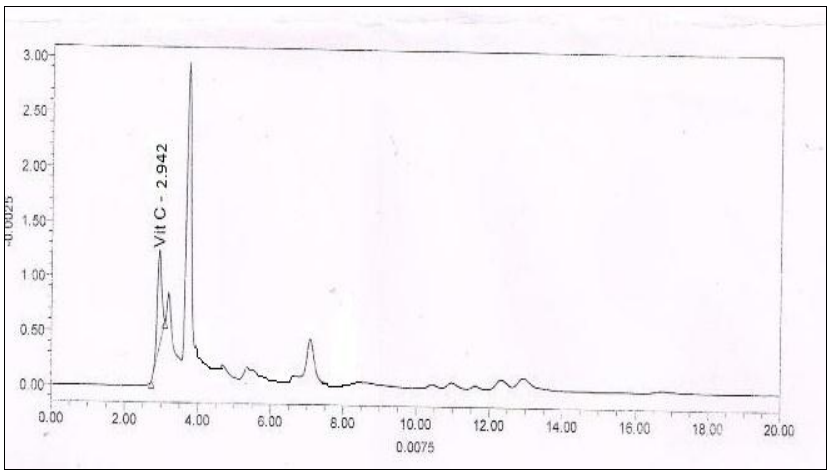
Peak area : 7326225

Height of peak : 894497

Concentration : 98.944mg/100g

Vitamin C content in wheatgrass powder was found to be 98.944 mg/100g.

2. Vitamin C in Wheatgrass juice



Graph 4: Calibration curve for Vitamin C in Wheatgrass juice.

λ –max : 240 nm

Retention time : 2.941

Peak area : 7325410

Height of peak : 894496

Concentration : 98.942mg/ 100g

Vitamin C content in wheatgrass juice was found to be 98.942 mg/100g.

From the above observation it is state that, the Vitamin C content in both Wheatgrass powder and Wheatgrass juice was found to be 98.944 mg/100g and 98.942 mg/100g respectively, i. e. only a slight variation was to be noted by analysing with hplc method.

CONCLUSION

Wheatgrass powder is more convenient than wheatgrass juice because.

1. In wheatgrass juice, leaching of water soluble tannin occurs in aqueous extract which make it black and will smell bad after course of time just what happens, in some river due to which they get blacken. Keeping juice for long time same result are shown by juice that it gets black. So in market always powder are recommended.
2. During drying sugar-rich glycosides can lose one or more sugar residue due to hydrolysis by glycosidase and due which aglycone part which is lipophilic gets separated from glycone part, powder form are recommended.
3. Chlorophyll content in both wheatgrass powder and wheatgrass juice varies, powder contains more chlorophyll contents than juice.
4. Vitamin C content in both wheatgrass powder and wheatgrass juice nearly same only slight variation is seen.
5. The above observation shows that, the wheatgrass powder have more convenience, stable, effective and easily available in market. Whereas, freshly prepared wheatgrass juice has not available easily in market, and not provided easily to patient immediately as compare to wheatgrass powder.
6. From the above results it could be concluded that wheatgrass should be consumed regularly and as per the bodily demand as they boost up the bodily mechanisms by one way or another way. The secondary metabolites like chlorophyll help in the maintenance of the primary metabolites. The ascorbic acid (Vitamin C) content is within the range of consumption, so are useful.
7. Results of our study indicate that use of wheatgrass can be beneficial in many disease conditions and inflammatory skin diseases.

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