Research Article

PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING OF WHEATGRASS JUICE *(TRITICUM AESTIVUM L.)*

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ABSTRACT

Wheatgrass (*Triticum aestivum* L.) belongs to the family Poaceae. Other plants belonging to this family include *Agopyron cristatum, Bambusa textilis, cynodon dactylon, Poa annua, Zea mays, Aristida purpurea*, etc. The present plant *Triticum aestivum* L. is mentioned in Ayurveda, herbal system of medicine and described as immunomodulator, antioxidant, astringent, laxative, diuretic, antibacterial and used in the acidity, colitis, kidney malfunction, swelling wounds and vitiated conditions of Kapha and Pitta. Wheatgrass is believed to be having property of optimizing blood sugar level. Now a days, its use as an antidiabetic agent is being popularized. But, still its scientific proof is not there. This project is just an attempt to provide evidence to its usefulness in management of diabetes mellitus.

Keywords: Wheatgrass (*Triticum aestivum* L.), Antidiabetic agent, Immunomodulator, Antioxidant, Astringent, Laxative, Diuretic.

INTRODUCTION

Triticum aestivum l. Belonging to family poaceae is a green commonly found herb in India, although its nativity has been lost today. This plant is believed to be having manifold pharmacological diversities in addition to its nutritional value which are yet to be explored. I decided to work on this plant to find out their usefulness to human being. The present works include screening of anti-hyperglycemic, antimicrobial and anti-oxidant properties of grass of *triticum aestivum* l. Along with its preliminary phytochemical evaluation.[1]

Need of Study[2]

- The modern medicines are very efficient in curing diseases but they are produces a number of side effects. On the contrary, the crude drugs are somewhat less efficient than modern medicines, but they are relatively free from side effects which made the traditional system of medicine to create the competition to modern day medicine.
- Synthetic drug research has yielded a number of medicines for the treatment of various health ailments, but at the same time there are many diseases like AIDS, Cancer, Asthma, Diabetes mellitus, Blood pressure etc. for which there is no definite and permanent cure available in modern system of medicine because of limitations in their use by its severe side effects. Therefore, there is an ever increasing need for efficacious, economic, safer medicinal agents producing permanent cure in the recent times. In this situation we could look to the plant kingdom for a remedy. India having rich resources of medicinal plants with rich knowledge of its medicinal value since ancient times but, there is no scientifically known rationality, thus, it would be ideal time and place for plant drug research.

Pharmacological Screening[3]

- **Anti-hyperglycemic activity on Alloxan induced diabetic Wistar rats**
  - Alloxan induced hyperglycemia in Wistar rats
  - Gliclazide used as standard against Wheatgrass juice.
- **Estimation of Liver Glycogen Level**
  - Animals in anti-hyperglycemic study were utilized again after scarification.
  - Liver tissue were isolated, (Gliclazide was used as standard again).
- **Antimicrobial activity**
  - Antibacterial study was done by using three bacterial strains.
  - S. aureus, P. aeruginosa, E. coli were used
  - Antifungal potency was analyzed by using Candida albicans.
  - Standards: Tetracycline (antibacterial activity), Ketoconazole (antifungal activity)
- **Anti-oxidant activity**
  - Antioxidant activity was analyzed by in vitro method.
  - Two methods were used viz. "DPPH free radical scavenging assay" and "Reducing power assay".

Chemo Texanomical Relationship[4]

Wheatgrass (*Triticum aestivum* L.) belongs to the family Poaceae. Other plants belonging to this family include *Agopyron cristatum, Bambusa textilis, cynodon dactylon, Poa annua, Zea mays, Aristida purpurea*, etc.

Plants belonging to the family Poaceae are mainly grasses. These grasses are included in traditional medicine system but yet have to be explored. Less scientific data is available on the plants belonging to the family Poaceae. However, few plants are studied and have proven scientifically. *Cynodon dactylon* is having proven anti-diabetic activity. *Zea mays* is another popular plant belonging to this family. *Zea mays* is multi-fascinated drug. It is mainly used in treatment of urinary tract infections. It is also having mild anti-bacterial and demulcent activities. *Triticum aestivum* L. is also an important plant belonging to this family. The plant is said to be having anti-cancer activity, according to traditional system of medicines.

The present plant *Triticum aestivum* L. is mentioned in Ayurveda, herbal system of medicine and described as immunomodulator, antioxidant, astringent, laxative, diuretic, antibacterial and used in the acidity, colitis, kidney malfunction, swelling wounds and vitiated conditions of Kapha and Pitta. Wheatgrass is believed to be having property of optimizing blood sugar level. Now a days, its use as an antidiabetic agent is being popularized. But, still its scientific proof is not there. This project is just an attempt to provide evidence to its usefulness in management of diabetes mellitus.

Figure 1: Herbarium of Wheatgrass (*Triticum aestivum* L.)

Preliminary Pharmacognostic Characteristics [5]

Macroscopy

Grass of Wheat berries

Botanical name: *Triticum aestivum* L.

Family: Poaceae

Description: Wheatgrass is young grass shoots of wheat berry. In appearance, wheatgrass is like any other grass. Culms are simple, hollow or pithy, glabrous, 1.2 m tall. Leaves flat, narrow, 20-38 cm long, 1.3 cm broad. Spikes long, slender, dorsally, compressed, somewhat flattened; rachis tough, not separating from spikelet's; 2-5 flowered, relatively far apart from stem, slightly overlapping, nearly erect, pressed closed to rachis; glumes keeled in upper half, firm, glabrous, shorter than lemmas; lemmas awned or awnless, less than 1.3 cm long; palea as long as lemma, remaining entire at maturity, caryopsis free threshing, soft or hard, red or white (hexaploid).

Table 1: Physical Characteristics of wheatgrass (*Triticum aestivum* L.) juice

Physical Constants	*Triticum aestivum* L.
Macroscopic Characteristics • Nature • Colour • Odour • Taste	 Grass Bright green/ Dark green Characteristic Acrid
Loss on Drying (%w/w)	21.1%

Qualitative Chemical Investigation

Table 2: Results of Phytochemical Investigation of Wheatgrass Juice

Name of the Test	Wheatgrass juice
Test for carbohydrates	
a) Molisch's test	+
b) Fehling's test	+
c) Benedict's test	+
Test for proteins	
a) Biuret test	+
b) Xantho protein test	+
c) Millons test	+
Test for Amino acids	
a) Ninhydrin test	+
Test for Alkaloids	
b) Dragendroff's test	+
c) Mayer's test	+
d) Hager's test	+
e) Wagner's test	+
Test for sterols	
a) Salkowski test	-
b) Liebermann reaction	-
Test for phenolics & tannins	
a) Ferric chloride test	-
b) Lead acetate test	-
c) Dil. HNO_3 test	-
Tests fixed oils and fats	
a) Saponification test	-
b) Stain test	-
Test for triterpenoids	
a) Liebermann Burchard's Test	-
b) Salkowski test	-
Test for glycosides	
a) Keller-Killiani Test	-
b) Baljet's Test	-
Test for saponins	
a) Haemolytic test	+
b) Foam test	+

(+) = Positive, (-) = Negative

Pharmacological Activity

Mechanism of diabetogenic action

1. Strecker reaction

The strecker reaction proceeds slowly and to provide an appreciable amount of the products in the β-cell, this reaction should take place intracellularly. In view of the short half-life of Alloxan in blood and the finding that it follows the distribution of mannitol being confined to the extracellular space. Diabetogenesis with the fact that several non-diabetogenic substances yields a positive strecker reaction.

2. Reaction with Sulfhydryl Groups

The hypothesis that alloxan may act by occupation or inactivation of –SH groups was advocated by Lazarow who showed that substances containing free sulfhydryl groups protect against Alloxan diabetes. These substances reduce Alloxan to dialuric acid, which is non-diabetogenic unless it is reoxidized to Alloxan.

3. Chelating action

Kadota has suggested that Alloxan diabetogenesis may be due to a combination of alloxan with zinc in the islet β-cell, which in turn may cause cell necrosis. This effect would be analogous to the mode of action of the chelating agents oxime and dithizone.

❖ **Drugs**

Alloxan (Loba chemie, Mumbai), Gliclazide (Panacea Biotec), Glucose estimation kit (Span diagnostics Ltd., Surat, India) were employed.

A) Evaluation of Anti-hyperglycemic activity[6]

Diabetes was induced in the animals by single injection of alloxan monohydrate (150 mg/kg, i.p.). It was confimed after 48 hours (on 3rd day). Animals found hyperglycemic (blood glucose level greater than 225 mg/dl) were divided into different groups. Control group received no drug treatment, standard group received Gliclazide (8 mg/kg, p.o) and test groups received wheatgrass juice (50 & 100 mg/kg, p.o) once a day up to 14th day of alloxan injection. Blood glucose was estimated in all groups on 15th day. Animals were sacrificed and their liver tissue was used for the estimation of glycogen.

B) Estimation of Liver Glycogen

Accurately weighed about 1.0 g of liver tissues, placed the tissues in calibrated centrifuge tube containing 2 ml of KOH (300 g/lit.), and heated in a boiling water bath for 20 min. with occasional shaking. The tubes were cooled in ice; 0.2 ml of saturated sodium sulphate was added and mixed thoroughly. Then glycogen was precipitated by adding 5 ml of ethanol and precipitate was removed by centrifugation. The precipitates were dissolved in distilled water (10 ml) with gentle warming. 1 ml of HCl (1.2 mol/lit.) was added. After placing marble on the top of each tube, tubes were heated in boiling water bath for 2 hours. After 2 hours 1 drop of phenol red indicator was added and neutralized with NaOH (0.5 mol/lit.). It was diluted to 5 ml with distilled water and the glycogen content was determined. Glycogen content was expressed as "gram/ gram" of liver tissue.

Evaluation of Antimicrobial Activity

Biological assay are divided in fours,

1. Diffusion assay or Cylinder plat or Cup- plat method.
2. Turbidimetric assay.
3. Metabolic response assay.
4. Enzymatic assay.

Table 3: Antimicrobial activity (Observation Table)

Sample No	*E. coli* (ATCC 25922)	*P. aeruginosa* (ATCC27853)	*S. aureus* (ATCC 25923)	*Candida albicans*
100 mg	No zone	No zone	No zone	--
200 mg	**5 mm**	**3 mm**	No zone	--
500 mg	**13 mm**	**11 mm**	**6 mm**	**9 mm**
Tetracycline	20 mm	20 mm	23 mm	--

Characterization

Gas Chromatography- Mass Spectroscopy Graph GC analysis

Figure 2: GC chromatogram

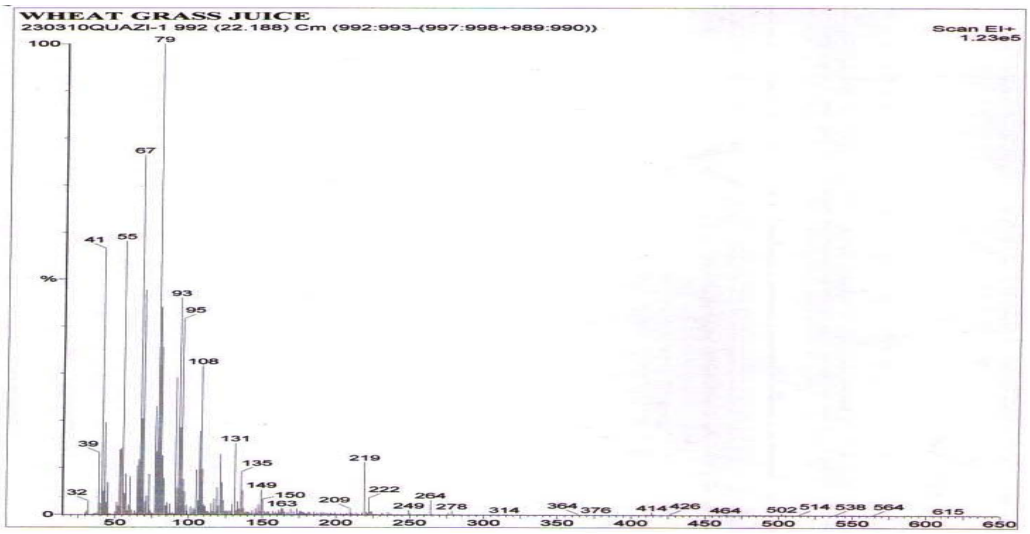

Figure 3: Mass Spectra

UV SPECTROPHOTOMETRIC ANALYSIS

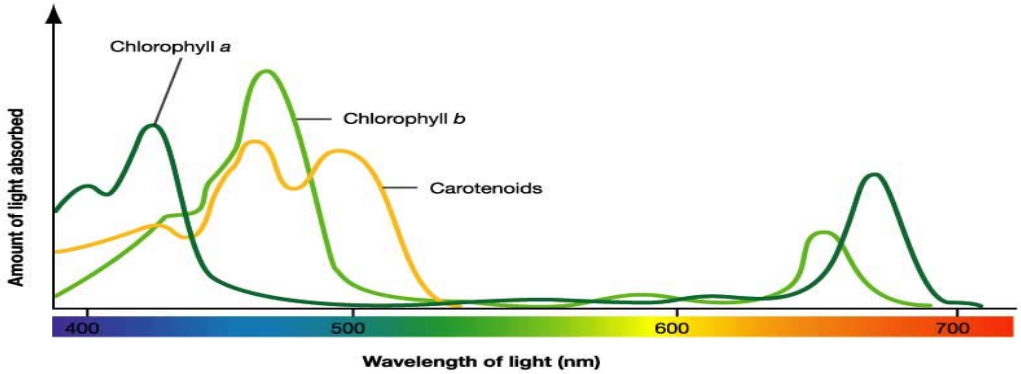

Figure 4: UV- Spectrophotometric characterization

Specifications: λmax for Chlorophyll a: 661.1 (std. 662); λmax for Chlorophyll b: 642.6 (std. (644); Solvent: 96% Methanol

HPLC ANALYSIS

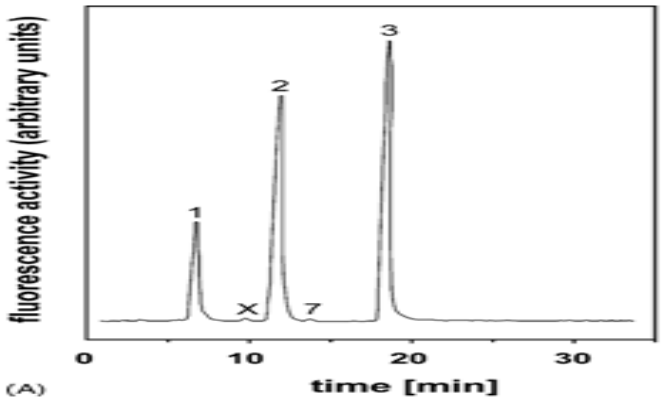

Figure 5: HPLC Chromatogram

Table 4: Observation of HPLC analysis of wheatgrass juice

No.	RT[min]	Area[mV*s]	Area %	TP	TF	Resolution
1	06.9921	300.1904	16.41	429.8	0.4655	0.0109
2	11.9819	720.5357	39.38	1180.9	1.2180	0.8029
3	19.8900	808.5355	44.20	1671.7	4.0988	0.9340

RESULTS AND DISCUSSION

Triticum aestivum L. Belonging to family Poaceae is a green commonly found herb in India, although its nativity has been lost today. This plant is believed to be having manifold pharmacological diversities in addition to its nutritional value which are yet to be explored. I decided to work on this plant to find out their usefulness to human being. The present works include screening of anti-hyperglycemic, antimicrobial and anti-oxidant properties of grass of *Triticum aestivum* L. along with its preliminary phytochemical evaluation.

This plant is cultivated and collected in the garden area of A.R.A. College of Pharmacy, Nagaon, Dhule. Herbarium of the plant specimen has been deposited at B. S. I., Pune, and the voucher specimen number **SMRN1** with reference number BSI/WC/Tech/2010/829 was given.

First of all Wheatgrass was cultivated in the garden area of our Institute, A.R.A. College of Pharmacy, Nagaon, Dhule. The seeds were sown after soaking them in good quality water for overnight (12 hrs.). The seeds were covered with the layer of the soil. The grass was cut on the 8^{th} day from the commencement of the procedure. The grass was cut from the root level. The juice of the same was prepared by using electric juicer (Grinder or juicer). The juice was then subjected for further studies.

In appearance the wheatgrass (*Triticum aestivum* L.) like any other grass. It is green in color, soothing and cooling. Wheatgrass is young grass shoots of wheat berry. The juice was prepared from 8 day old grass of the plant *Triticum aestivum* L. by using electric grinder with little aid of water for the lubrication. Extraction using different organic solvents is avoided considering solubility of chlorophyll.

Standardization of plant extract (Juice) was done with the help of extractive values (water and methanolic) and loss on drying (LOD).

Table 5: Phytochemical constituents of wheatgrass juice

Sr.No	Phytochemical constituents	Observations
1.	Alkaloids	+
2.	Flavanoids	_
3.	Carbohydrate	+
4.	Saponins	+
5.	Triterpenes	_
6.	Phytosterols	_
7.	Tannins	_
8.	Glycosides	_
9.	Fixed oil & fats	_
10	Phenolic compound	_
11	Gum & mucilages	+
12	Proteins & amino acid	+

Water soluble extractive value was found to be greater than alcohol soluble extractive value in the experiment. The reason behind that is chlorophyll content of wheatgrass juice which is about 70%. Chlorophyll is water soluble. This fact resulted in water soluble extractives to be about 71.1% and the alcohol soluble fraction was found to be just 9.2%. The wheatgrass juice was also subjected for LOD study. The total reduction in weight was found to be 24.86%. At the end of the procedure the material obtained is anhydrous wheatgrass powder.

Anti-hyperglycemic activity was performed by *in vivo* method involving Wistar rat 21 day model. The result obtained was expressed in terms of blood glucose level (mg/dl) and compared with standard drug Gliclazide. Among the two doses, higher dose (100 mg, p.o.) showed significant anti-hyperglycemic activity.

Liver glycogen estimation was done by making use of same Wistar rats that are used in Anti-hyperglycemic activity. The rats in each group are sacrificed and effect of wheatgrass juice on liver glycogen level was studied. The results are satisfactory and we can say that wheatgrass can prevent occurrence of hypoglycemic shocks. The values of liver glycogen level were noted and expressed in the terms of "mcg/gm" of liver tissue.

CONCLUSION

Antioxidant activity of the wheatgrass juice was compared with the standard drug ascorbic acid. From the graph of percent inhibition and IC_{50}, it shows that wheatgrass juice is having significant antioxidant activity that is comparable to the standard drug ascorbic acid. Two methods were used for evaluation of antioxidant property of wheatgrass juice namely DPPH model and reducing power assay.

DPPH assay involves the use of DPPH (Diphenyl picryl hydrazyl). Its reaction rate directly relates to the antioxidant activity. Reducing power assay assess antioxidant activity by the help absorption criteria. Antioxidant property is directly proportional to the UV absorption. Higher the absorbance of the reaction indicated greater the reducing potential.

Instrumental Characterization of Wheatgrass (spray dried powder of juice) confirmed the presence of Chlorophyll which is believed to be pharmacologically active component in wheatgrass as and antidiabetic agent.

- λmax for chlorophyll A is found to be 661.1 while 642.6 for chlorophyll B.
- GC-MS data showed peaks related to compounds which are degradation products of Chlorophyll.
- HPLC analysis revealed the probable presence of Chlorophyll A & B as well.

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