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Original Article

Pharmacognostic, phytochemical investigation & pharmacological study of young leaves of *Triticum aestivum* Linn.

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ABSTRACT

The aim of the work is to perform the pharmacognostic study of the leaves of plant Triticum aestivum Linn. Family Poaceae, commonly known as 'Wheatgrass'. It is cultivated on large scale all over India and also occasionally cultivated in garden. For the present study samples of the Wheatgrass leaves were collected over a specific period of nine days. The drug was cultivated with specific type of hybrid seeds obtained from most reputed institution of India and were scrupulously analysed. For standardization of the herbal drug morphological, phytochemical, physicochemical and microscopical examination was done. The leaves grown were found to be lax, cauline, flat, 0.6 to 0.25 inches (4 to 6 mm) wide, 6-9 inches long and green in colour. The chemical compositions of the leaves are proteins, flavonoids, alkaloids, glycosides, terpenoides, saponins, fibres, tannins and phenolic compounds. The plant juice is the mainly used for cancer alignment and main source of medicine used along with Basil, mint & Neem leaves to reduce toxicity & cancer cells.

1. INTRODUCTION

The plant *Triticum aestivum Linn.* belonging to the family Poaceae can be used for different liver ailments, to help prevent cancer, tooth decay, skin problems such as eczema and psoriasis [7]. It is also claimed to reduce hair from graying, improves digestion, reduces high blood pressure as it enhances the capillaries, support the growth of lactobacilli and can remove heavy metals from the body [8,9,10] It is found to improve hematological toxicity related to chemotherapy in breast cancer patients, chemoprevention of mouse skin carcinogenesis induced by DMBA and croton oil in association with oxidative status, it reduces the frequency and requirement of blood transfusions in thalassemia major [1,2,3,4]. Various useful attempts have been made for the pharmacognostic study of the drug [5,6]. This work deals with the ninth day young wheatgrass when the plant is at its full nutritional power, called the jointing stage (Figure 1 and 2) and has tried to explore the microscopical elucidation with more descriptive interpretation and besides this the various distinct longitudinal sections have been used to systematize pharmacognostic evaluation of wheatgrass.

2. MATERIALS AND METHODS

2.1 Preparation of *Triticum aestivum* powder

The ninth day grass of *Triticum aestivum* was cultivated, collected and chopped with the help of knife. It was dried in shade and then powdered with a mechanical grinder. The powder was passed through sieve no.40 and stored in a labelled air tight container.

2.2 Macroscopic studies

The fresh herb was subjected to macroscopic studies which comprised of organoleptic characters of the drugs viz. colour, odour, appearance, leaves size, taste and texture.

Fig. 1. *Triticum aestivum* (Wheatgrass)

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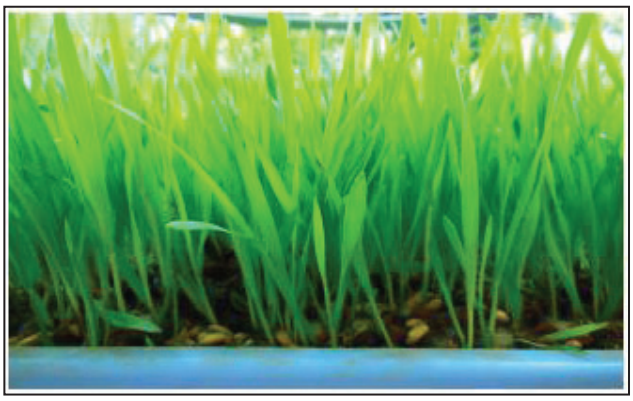
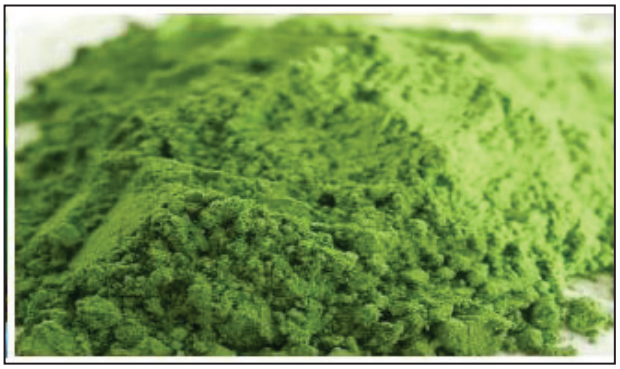
2.3 Microscopic studies

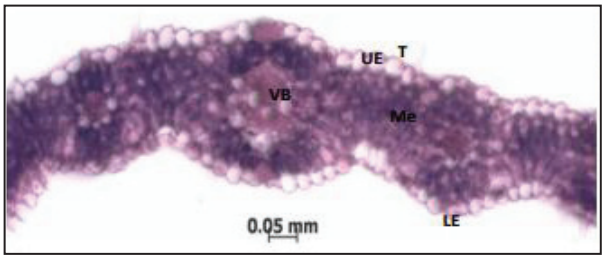
Qualitative microscopic evaluation was carried out by taking transverse and longitudinal sections of fresh leaves. Free hand sections of the fresh leaves were boiled with chloral hydrate to remove all the colouring matter. The sections were transferred and mounted (glycerine) on a slide and a cover slip was placed over it [11,12]



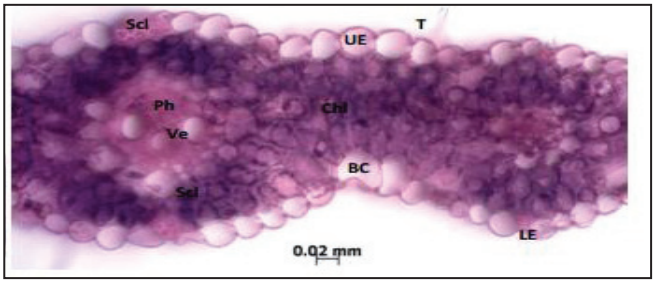
Vascular bundle



Godhurna patna powder



TS of lamina passing through midrib



Portion enlarged

Fig. 2. Microscopic study of *Triticum aestivum*

2.4 Physicochemical studies

Physicochemical studies include ash value and extractive value to determine the quality and purity of the powder of plant of Triticum aestivum. [13,14]

2.4.1 Ash values:

2.4.1.1 Total ash value: Accurately weighed 2gm of air dried sample were taken in a tarred silica dish and incinerated at a temperature not exceeding 450°C until free from carbon, then cooled and weighed. Percentage of ash value was calculated with reference to the crude air dried drug.

2.4.1.2 Acid insoluble ash: Ash was boiled with 25ml of 2 M HCl for 5 min, insoluble matter was collected in a Gooch crucible in an ash less filter paper, washed with hot water, ignited, cooled in desiccators and weighed. Percentage of acid insoluble ash was calculated with reference to the air dried drug.

2.4.1.3 Water soluble ash: Ash was boiled for 5 min with 25ml of water, insoluble matter was collected in a Gooch crucible in an ash less filter paper, washed with hot water and ignited for 15 min at a temperature not exceeding 450°C. Weight of insoluble matter was subtracted from the weight of the ash; the difference in weight represents the water soluble ash. Percentage of water soluble ash was calculated with reference to the air dried drug.

2.4.2 Extractive Values

2.4.2.1. Water soluble extractives: 4gm of air dried plant material was macerated with 100ml of water in a closed flask, shaking frequently during the first 6hr and allowed to stand for 18 hr. Thereafter it was filtered rapidly taking precaution against loss of water. 25ml of filtrate was evaporated to dryness in a tarred flat bottom shallow dish dried at 105°C and weighed. Percentage water soluble extractive was calculated with reference to the crude air dried plant material.

2.5 Preliminary phytochemical screening

The alcoholic and aqueous extracts of *Triticum aestivum* were subjected to preliminary phytochemical screening to determine the presence of phytoconstituents. Screening was carried out on both the *Triticum aestivum* extracts to determine the active principles or secondary plant constituents. Two milliliters of each extract were measured into a test tube for each of the tests and concentrated by evaporating the extract in a trough. Tests were carried out for carbohydrates, reducing sugars, tannins, polyphenols, lipids, flavonoids, ketones, alkaloids, steroids and triterpenes.

2.5.1 Alkaloids: Mayer's test. Alkaloids gave cream colour precipitate with Mayer's reagent (potassium mercuric iodide solution). Dragandroff's test. Alkaloids gave reddish brown precipitate with Dragandroff's reagent (potassium bismuth iodide solution).

Wagner's test. Alkaloids gave a reddish brown precipitate with Wagner's reagent (solution of iodine in potassium iodide).

Hager's test. Alkaloids gave yellow colour precipitate with Hager's reagent (saturated solution of picric acid).

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2.5.2 Glycosides: General test for the presence of glycosides Part A: 200mg of the drug was extracted by warming in a test tube with 5ml of dilute (10%) sulphuric acid on a water bath at 100°C for 2 min, centrifuged, pipetted off supernatant. The acid extract was neutralized with 5% solution of sodium hydroxide (noting the volume of sodium hydroxide added). Fehling's solution A and then B were added until solution became alkaline (tested with pH paper) and heated on a water bath for 2 min. Noted the quantity of red precipitate formed and compare with that formed in Part-B.

Part B: 200mg of the drug was extracted using 5 ml of water instead of sulphuric acid. After boiling, equal volume of water to that of sodium hydroxide used in the above test was added. 0.l ml of Fehling's solution A and B were added until solution became alkaline (tested with pH paper) and heated on water bath for 2 min. The quantity of red precipitate formed was noted. The quantity of precipitate formed in Part-B was compared with that formed in Part-A. If the precipitate in Part-A was greater than in Part-B then Glycoside may be present. Since Part-B represents the amount of free reducing sugar already present in the crude drug. Whereas Part-A represents free reducing sugar plus those related on acid hydrolysis of any sides in the crude drug.

2.5.2.1 Saponin glycosides: Froth test: Placed 1ml solution of drug in water in a semi micro tube, shaken well and noted the stable froth.

2.5.2.2 Anthraquinone glycosides: Borntrager's test: Boiled test material with 1ml of dilute sulphuric acid in a test tube for 5 min (anthracene glycosides were hydrolyzed to aglycone and sugars by boiling with acids) centrifuged or filtered while hot (if centrifuged hot, the plant material can be removed while anthracene aglycones are still sufficiently soluble in hot water, they are however insoluble in cold water), pipetted out the supernatant, cooled and shake with an equal volume of dichloromethane (the aglycones will dissolve preferably in dichloromethane) separated the lower dichloromethane layer and shaked with half its volume with dilute ammonia. A rose pink to red colour was produced in the ammonical layer (aglycones based on anthroquinones give red colour in the presence of alkali).

Modified Borntrager's test: Boiled 200 mg of the test material with 2ml of dilute sulphuric acid, 2ml of 5% aqueous ferric chloride solution for 5 min and continued the test as above. As some plant contain anthracene aglycone in a reduced form, ferric chloride was used during the extraction, oxidation to anthroquinones took place, which showed response. Borntrager's test.

2.5.2.3 Cardiac glycosides: Keller Killiani test (Test for deoxy sugars): Extracted the drug with chloroform and evaporated it to dryness. 0.4ml of glacial acetic acid was added which contained a trace amount of ferric chloride and was transferred to a small test tube. Carefully 0.5ml of concentrated sulphuric acid was added along to the side of the test tube, blue colour appeared in the acetic acid layer.

2.5.3 Tannins and phenolic compounds

2.5.3.1 Gelatin test: Extract with 1% gelatin solution containing 10% sodium chloride gave white precipitate. Ferric chloride test. Test solution gave blue green colour with ferric chloride.

2.5.3.2 Vanillin hydrochloride test: Test solution when treated with few drops of vanillin hydrochloride reagent gives purplish red colour.

2.5.3.3 Heavy metal test: Tannins got precipitated in the solution when treated with heavy metals.

2.5.3.4 Alkaline reagent test: Test solution with Sodium hydroxide solution gave yellow to red precipitate within short time. Mitchell's test. With iron and ammonium citrate or iron and sodium tartarate, tannins gave a water soluble iron tannin complex, which was insoluble in solution of ammonium acetate.

2.5.4 Flavonoids Shinoda test (Magnesium hydrochloride reduction test): To the test solution, few fragments of magnesium ribbon were added and concentrated hydrochloric acid was added drop wise, pink scarlet, crimson red or occasionally green to blue colour appeared after few minutes.

2.5.5 Zinc hydrochloride reduction test: To the test solution, a mixture of zinc dust and concentrated hydrochloric acid were added. Red colour obtained after few minutes.

2.5.6 Millons test: Test solution was mixed with 2 ml of Millons reagent (mercuric nitrate in nitric acid containing traces of nitrous acid), white precipitate appeared, which turned red upon gentle heating.

2.5.7 Ninhydrin test: Amino acids and Proteins when boiled with 0.2% solution of Ninhydrin (indane 1, 2, 3 trione hydrate), violet colour appeared [16,18]

3. RESULTS AND DISCUSSION

The plant of *Triticum aestivum* is an indigenous herb which was chosen for this study. It belongs to the family Poaceae. The scanty availability of information on this plant facilitates the study on it since ages this plant is being used for its medicinal value. Various useful attempts have been made for the pharmacognostic study of the drug. This attempt is made to study in detail the transverse as well as the longitudinal section of the drug so that the specific variety of the drug can be identified and collected at the specific time for its various important chemical constituents so that the drug can be used effectively to its full potential.

Table 1. Physicochemical parameter of *Triticum aestivum* (Wheat Grass)

Sample Identity	Leaves
Moisture Content	4.05
% Total Ash	7.5
% Acid insoluble ash	2.3
% Water soluble ash	4.0
% Water soluble Extractive value	2.5
% Alcohol soluble Extractive value	3.75

Table. 2 Preliminary phytochemical screening of *Triticum aestivum* (Wheat Grass)

S. No.	Parameters	Methanol	Ethyl acetate	Chloroform	Aqueous
1.	Carbohydrates	+	+	+	+
2.	Proteins	+	-	-	+
3.	Alkaloids	+	-	-	+
4.	Flavonoids	+	-	-	-
5.	Tannins	-	-	-	+
6.	Phenols	-	-	-	+
7.	Saponins	-	-	-	+
8.	Glycosides	+	-	-	+
9.	Steroids	+	-	-	-
10.	Terpenoids	+	-	-	-

(+) = indicates presence of compounds

(–) = indicates absence of compounds

4. CONCLUSION

Preliminary phytochemical as well as macroscopic and microscopic characteristics of the plant were studied for quality control of raw drug. The plant of *Triticum aestivum* exhibits a set of diagnostic characteristics which will help to supplement information in regard to its identification parameters assumed significantly in the way of acceptability in present scenario of lack of regulatory laws to control the quality of drug. It has been concluded from this study that estimation is highly essential for raw drugs or plant parts used for the preparation of compound formulation of drug. The periodic assessment is essential for quality assurance and safer use of herbal drugs. The drug is promising if the punctiliously selection of variety, its time of collection and identification is done.

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