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Biochemical Analysis of Phenolics Compounds Accumulated During Developing Stages of Wheatgrass

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ABSTRACT

The current study was carried out to evaluate the accumulation of phenolic compounds in distinct developmental stages of Wheatgrass (*Tritium aestivum*). The wheatgrass was grown in the container/tray in the standard condition in the laboratory space provided by the department. Overnight soaked seeds were then sowed in the soil containers for 25 days. The accumulation of phenolics compounds was qualitatively and quantitatively checked in the leaves at 5, 10,15,20,25 days. Phenolics compounds were sequentially extracted in methanol, n-hexane, and acetone. For qualitative analysis, various biochemical tests were carried out and phenolics compounds were quantitatively estimated by Folin-Ciocalteu reagent. Among three solvents methanol extract was found to accumulate phenolics compounds in increasing concentration at various developmental stages i.e. 5th days - 0.7135 mg/ml, 10th days 1.2614 mg/ml, 15th days- 1.1244 mg/ml; 20th days - 1.8093 mg/ml and 25th days - 4.0693 mg/ml. On the qualitative check, methanol extract was found to be positive for tannins, terpenoids, and glycosides. These phenolics could be analyzed for antidiabetic and antimicrobial activities are the future perspective of this study.

Keywords- Wheatgrass, Phenolics compounds, Qualitative analysis.

I. INTRODUCTION

Diabetes mellitus (DM) is a condition that impairs the body ability to process blood glucose, otherwise known as blood glucose. Diabetes currently affects more than 62 million Indians, which is more than 7.1% of adult population. Diabetes can lead to a build of sugar in the blood, which can increase the risk of dangerous complication including stroke and heart disease. There are three major types of diabetes (type1, type2 and gestational diabetes) in that type1 diabetes this type occurs when body fail to produce insulin and people with type1 diabetes are insulin dependent, which mean they must take artificial insulin daily to stay live. Type2 diabetes affects the way of body use to insulin. While the body still makes insulin the cell in the body do not respond to it. Gestational diabetes is occurring in women during pregnancy when body can becomes less sensitive to insulin (https://www.medicalnewstoday.com/articles/323627.php).

Plant have major source of drug for the treatment of diabetes mellitus in Indian system of medicine and other ancient systems in the world.

Wheat is an important source of carbohydrates [1] Globally, it is the leading source of vegetable protein in human food, having a protein content of about 13%, which is relatively high compared to other major cereals [2-3] but relatively low in protein quality for supplying essential amino acids [4]. When eaten as the whole grain, wheat is a source of multiple nutrients and dietary fiber [5]. Wheatgrass is the newly developed leaves of the wheat plant (*Triticum aestivum*), consumed as a food, drink, or dietary supplement. With antioxidant efficacy, wheat grass showed antidiabetic activity as reported in folk medicine [5].

There are around 300,000 species of higher plants listed on the planet, which synthesize a wide range of chemicals of manifold structure and class (more than 200,000 isolated and identified individual entities) these compounds can further classify into primary and secondary metabolites [7-8]. Sugar, fatty acids, amino and nucleic acid, as well as the chemicals necessary for growth and development of plants are considered as primary metabolites [9]. Among secondary metabolites which plays important role in plant defense, phenolic compounds are divers and from simple phenolic to highly polymerized complex phenolic compounds contain benzene ring, with one or more hydroxyl substituents [10]. Phenolics compounds are prominent secondary metabolites occurs in plants and majority of them possess biological activities beneficial to host plants as well as to humans as therapeutic agents [11-13]. The phytochemicals specifically phenolic compounds from some selected plants are demonstrated to have anti-diabetic activity by inhibiting human digestive amylase which could be therapeutic strategy for the management of DM [14]. Beside this phenolic compounds, including phenolic acids and flavonoids,

could promote health benefits by limit the risk of metabolic syndrome and problems the related to DM [15].

The current study was aimed to evaluate qualitatively and quantitatively the accumulation of phenolic compounds at different development stages of Wheatgrass.

II. METHODOLOGY

Chemical

Ethanol, Methanol, Hexane, Acetone, Dimethyl sulfoxide, Sodium Carbonate, Folin-Cioealteu Reagent, Gallic Acid, Starch and Lugol's Reagent. All other chemicals were analytical grade.

Growth condition and preparation of samples

The Wheatgrass was grown in the container/tray in the laboratory area of institute as per requirements. Overnight soaked *T. aestivum* seeds were equally spread over soil. Sprinkled small quantities of water daily until the required growth/days (5,10,15,20,25 days) and kept in to sun light for 3-4 hours daily. On the 5^{th},10^{th},15^{th}, 20^{th} and 25^{th} days from the germination, the wheat grass was cut 1cm above the surface of soil. As need of continuous harvesting of fresh grass, pots were similarly planted at one-day of interval.

Figure 1: Wheat Grass grown under laboratory condition.

Ten grams of harvested fresh grass were cut into further small pieces. Then the juice of grass was prepared by grinding it in to mortar pestle [16]. The crude phenolic compound samples were prepared in methanol, acetone and n-hexane as shown in following workflow (**Figure 2**).

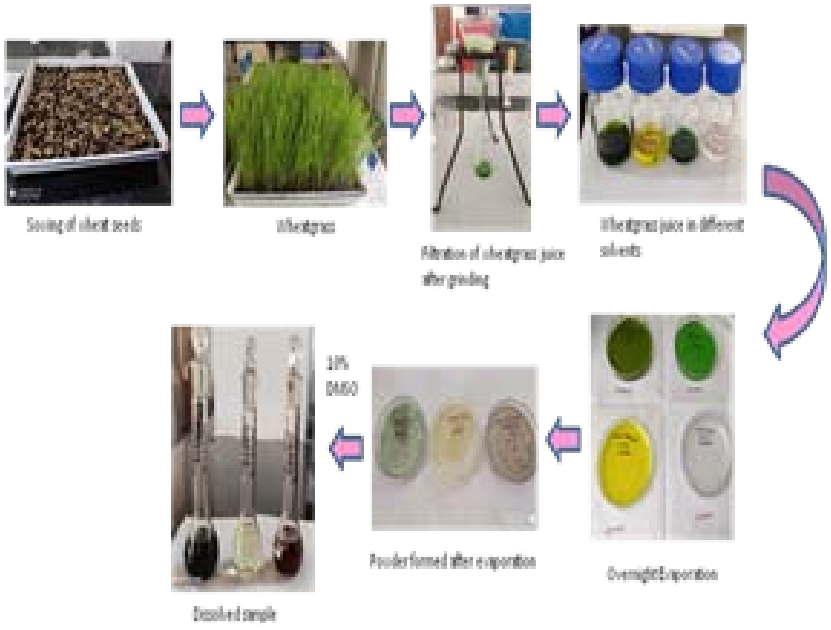

Figure 2: Workflow of samples preparation.

Quantitative estimation of phenolic compounds

Quantitative analysis of total phenolics in extract was determined with the Folin-Ciocalteu reagent. Standard phenolic compound used for the analysis was gallic acid (GA). The concentrations were expressed in mg GAeq/ml. Concentration of 0.00, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3 and 0.35 mg/ml of gallic acid were prepared in distilled water. The standard graph was obtained for Y= 139.1335x; R^2= 0.9976 **(Figure 3)**.

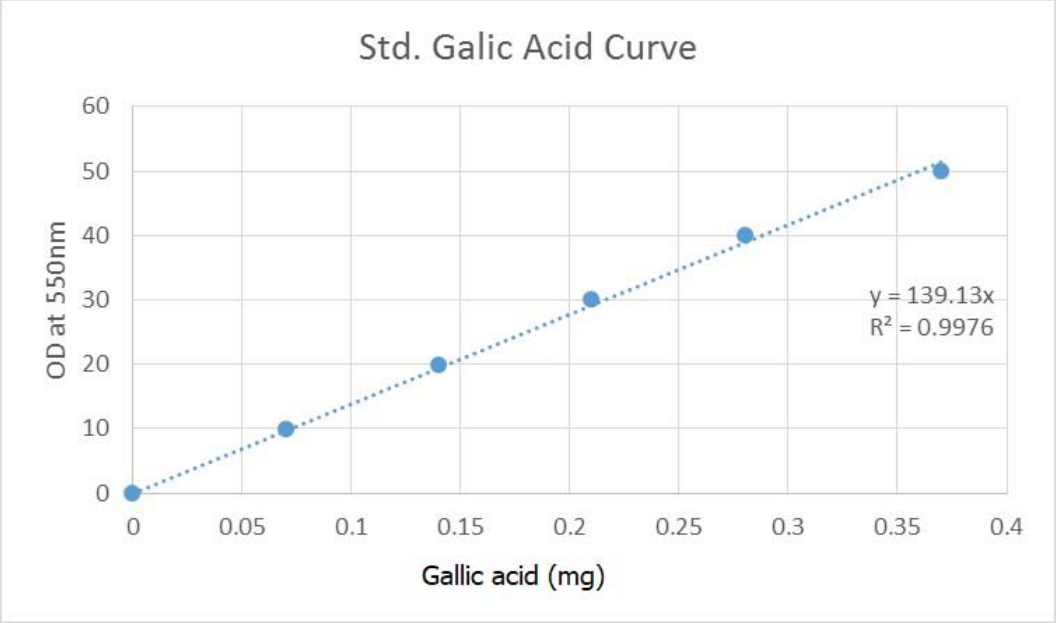

Figure 3: Standard curve of Gallic acid

With the help of this standard graph the concentration of phenolic compounds were estimated using following equations.

For Methanol

$X= \frac{given\ M.E. \times 1000}{20}$

Where X=calculated M.E.value

FOR HEXANE

$X= given\ H.E. \times 5$

Where, X=calculated H.E.value

FOR ACETONE

$X= given\ A.E. \times 10$

Where, X=calculated A.E.value

Qualitative analysis of primary and secondary metabolites

Qualitative analysis of primary and secondary metabolites was carried out using following tests.

Test for carbohydrate

1. Molish's Test

3.75 grams nephthol was dissolved in 99% ethanol. To this reagent with crude extract, few drops of Conc. H_2SO_4 were added. The formation of a purple or a purplish red ring at the point of contact between H_2SO_4 confirmed the presence of carbohydrate.

2. Fehling's test

34.66 grams copper sulphate was dissolved in 500 ml (Fehling's A). 125grams potassium hydroxide and 173gm of sodium potassium tartarate were added in 500ml distilled water (Fehling's B). Equal volume of solution A and solution B were mixed to obtain Fehling's reagent. For test 1ml of crude samples were taken in dry test tubes and 1ml of Fehling's reagent were added to test tubes. The test tubes then kept in boiling water bath for 5 min. Reddish brown precipitate indicates positive test.

3. Benedict's test

100 ml of Benedict's reagent was prepared by taking 1.73grams of copper sulfate pentahydrate, 10grams of sodium carbonate and 17.3grams of sodium citrate in distilled water. 1ml each crude samples is taken into clean test tube. To this 2ml Benedict's reagent were added. The test tubes were kept in boiling water bath for 3-5 min. immediate formation of reddish precipitated indicated presence of reducing sugar.

Test for alkaloids

1. Mayer's Test

Mayer's reagent was prepared by dissolving 1.36 grams mercuric chloride and 5grams potassium iodide in 100ml distilled water. 1 ml each crude samples were taken into different test tubes and few drops of Mayer's reagent were added. The white or cream colour indicates presence of alkaloids.

2. Wagner's Test

To prepare Wagner's reagent 2 grams iodine and 6 grams potassium iodide were dissolved in 100ml

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distilled water. 1 ml each crude samples were taken into different test tubes and few drops of Wagner's reagent were added. Reddish brown precipitate indicates presence of alkaloids.

Test for steroids

The presence of the plant steroid in the test sample was carried out using following method. About 1ml crude samples were mixed with 10 ml chloroform and equal volume of sulfuric acid was added. Positive test indicates upper layer in test tube was turn into red and sulfuric acid layer showed yellow with green fluorescence.

Test for Phenol and Tannin

1. Lead acetated test

The crude samples were treated with few drops of 1% lead acetate solution. Observed the yellow or red precipitated.

2. Ferric chloride test

The crude samples were treated with 2ml of $FeCl_3$. The blue or black precipitates were observed.

Test for terpenoids

For terpenoids test, crude samples were treated with 2ml of chloroform and 1ml of concentrated H_2SO_4. The reddish brown colour is observed in positive test tubes.

Test for flavonoids

1-5 drops of concentrated HCI were added to little amount of crude extracts l. Immediate development of red colour indicted presence of flavonoids.

Test for glycoside

Small amount of plant extracts were taken in 1ml water in test tube and few drops of aqueous NaOH added. Yellow coloration indicates the presence of glycosides.

Test for saponins

The plant crude extracts were treated with sodium bicarbonate. Honey comb froth formation is indicated the presence of saponins.

All qualitative tests were carried out for 5, 10, 15, 20 and 25 days of samples of methanol, acetone, and n-hexane extracts.

III. RESULTS AND DISCUSSION

Quantitative estimation of phenolic compounds

The Folin-Ciocalteu reagent (FCR) or Folin's Phenol reagent or Folin-Denis reagent is a mixture of phosphomolybdate and phosphotugstate used for the colorimetric in vitro assay of phenolic compounds. In present study, we extracted phenolic compounds in three different solvents i.e. methanol, n-hexane and acetone and estimated amount by FCR method. **Figure 4** shows the amount of phenolic compounds at different developmental stages i.e. Stage 1= 5 days, stage 2=10days, stage 3=15 days, stage 4= 20 days and 5= 25 days of samples of methanol, acetone, and n-hexane extracts respectively.

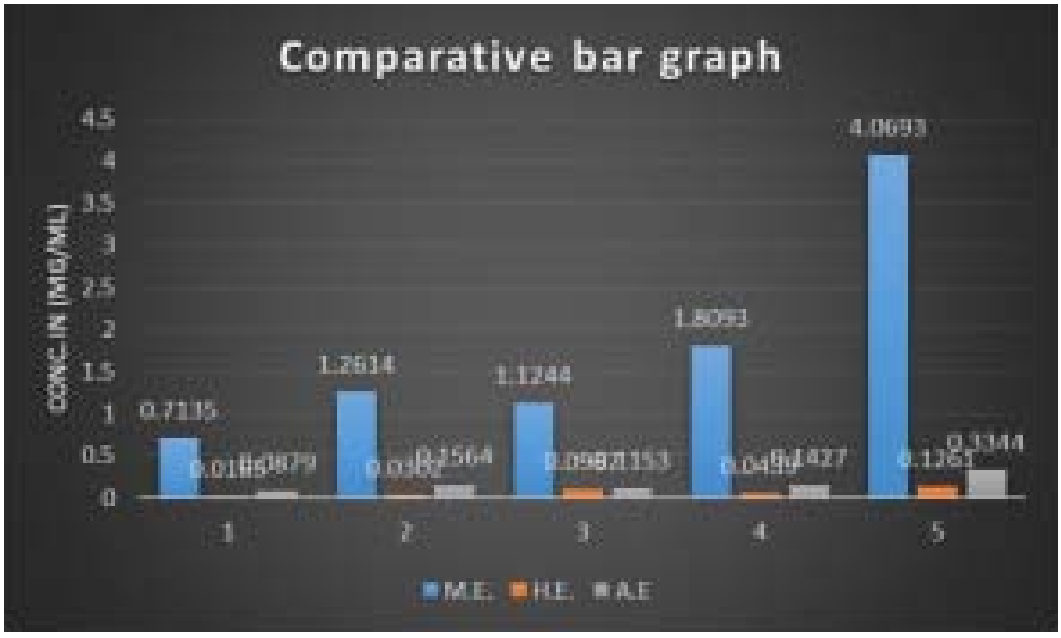

Figure 4: Quantitative analysis of phenolic compounds.

Among three solvents methanol extract was found to Among three solvents, methanol accumulated phenolics compounds in increasing concentration at various developmental stages i.e. 5th days - 0.7135 mg/ml, 10th days 1.2614 mg/ml, 15th days- 1.1244 mg/ml; 20th days - 1.8093 mg/ml and 25th days - 4.0693 mg/ml. On the qualitative analysis, methanol extract was found to be positive for tannins, terpenoids, and glycosides.

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Table 1: Qualitative analysis of the primary and secondary metabolites of wheatgrass.

Test	Methanol Extract Days					Hexane Extract Days					Acetone Extract Days				
Test for carbohydrates	5	10	15	20	25	5	10	15	20	25	5	10	15	20	25
a. Fehling's test	+	+	+	+	+	-	-	+	-	+	-	-	-	-	-
b. Benedict's test	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-
c. Molish's test	+	-	-	+	+	-	+	+	-	-	+	+	-	-	-
Test for alkaloids															
a. Meyer's test	+	+	-	+	+	-	-	-	-	-	-	-	-	+	+
b. Wagner's test	+	+	-	+	+	-	-	-	-	-	-	-	-	+	+
Test for steroid															
a. Salkowski test	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-
Test for phenolics and tannin															
a. Ferric chloride	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
b. Lead acetate	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Test for terpanoid															
a. Salkowski test	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
b. Liebermann burchard's	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Test for flavonoid															
a. Flavonoid test	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Test for glycoside															
a. Baljet's test	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-
Test for saponins															

From Table 1, it can be reported that wheatgrass accumulates substantial level of primary and secondary metabolites namely carbohydrates, flavonoids, saponins, polyphenols, and alkaloids during its development stages. Most of these metabolites were successfully extracted in methanol with some were also reported in solvent extracts with n-hexane and acetone. Primary metabolites are well known for its role in the processes of photosynthesis and respiration, while secondary metabolites are important for plant defense [17].

The analyses of the plants for its phytoconstituents are essential to exploit for commercial interest in both research institutes and drug discovery for the treatment of various diseases. Thus, we aimed to analyse these phytoconstituents at various development

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stages of wheatgrass which is reported to have anti-diabetic potential We hope that the present study will be helpful in the establishment of anti-diabetic potential of wheatgrass .

IV. CONCLUSION

The current study reports that methanol extract accumulates substantial amounts of phenolic compounds at different stages of development of wheatgrass. Phenolic compounds namely tannins, terpenoids, and glycosides were accumulated in developing stages and could be analyzed for antidiabetic and antimicrobial activities are the future perspective of this study.

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