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Biochemical studies on wheat (*Triticum aestivum* L.)

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Abstract
Wheat (*Triticum aestivum* L.) plays a key role in traditional health care system for human and animals. Seeds from wheat crops possess a significant amount of phytochemicals. In Present study qualitative and quantitative phytochemical analysis of wheat extract was made. Various bioactive compounds like alkaloids, saponins, glycosides, terpenoids, steroids, flavonoids and tannins were detected. The presence of such compounds in plants might be responsible for their curative effect. HPTLC studies of alcoholic extracts were carried out. HPTLC chromatogram shows many colored spots which indicate the presence of biomolecules in the drug. The findings of the study reveals a strong hope for development of more chemotherapeutic agents.

Keywords: bioactive compounds, carbohydrate, quantitative analysis, saponin, HPTLC

1. Introduction
Wheat is a major crop and an important component of the human diet, particularly in developing countries. Wheat varieties and cultivars are grown for particular characteristics that are suitable for specific products. For example, hard wheat flour characterized by high levels of gluten is used for bread and fine cakes, whereas durum wheat flour is used for macaroni, spaghetti, and other pasta products [1]. Wheat quality has traditionally been judged on the basis of functionality, mostly on gluten content and color, and, to a lesser extent, nutritional value. Color is an important quality parameter with regard to pasta production and is determined in part by carotenoids as well as other factors determined by the genetic makeup of the variety [2]. Wheat belongs to the family Triticeae (=Hordeae) in the grass family Poaceae (Gramineae). Common wheat (*Triticum aestivum*), also known as bread wheat, is a cultivated wheat species. About 95% of the wheat produced is common wheat. Wheat (*Triticum aestivum* L. em Thell.) is the first important and strategic cereal crop for the majority of world's populations. It is the most important staple food of about two billion people (36% of the world population). Worldwide, wheat provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally. It exceeds in acreage and production every other grain crop (including rice, maize, etc.) and is therefore, the most important cereal grain crop of the world, which is cultivated over a wide range of climatic conditions and the understanding of genetics and genome organization using molecular markers is of great value for genetic and plant breeding purposes [3-5].

Classification of wheat
Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Liliopsida
Order:	Poales
Family:	Poaceae
Subfamily:	Pooideae
Tribe:	Triticeae
Genus:	Triticum
Species	*T. aestivum*

Importance and Uses of Wheat
Grain-based foods, like those produced with wheat, provide complex carbohydrates, that are the best fuel for our bodies, are low in fat, high in fiber, and provide vitamins, especially the 4 key B vitamins, Thiamin, Riboflavin, Niacin, and Folic Acid, as well as iron. Wheat provides us with a nutritious and delicious supply of breads, pasta, cereals, crackers, bagels, and many other food products that has wheat as an ingredient.

Wheat is used in many other products that we use Straw particle board (wood) - used in kitchen cabinets, Paper, Milk replacer, Hair conditioners, Biodegradable golf tees, Adhesives on postage stamps, Water-soluble inks, Medical swabs, Charcoal, Biodegradable plastic eating utensils etc.

Young cereal plants were valued in ancient times. The plant *Triticum aestivum* can be used for different liver ailments, to help prevent cancer, tooth decay, skin problems such as eczema and psoriasis [6]. 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 [7-10]. It is found to improve hematological toxicity related to chemotherapy in breast cancer patients, it reduces the frequency and requirement of blood transfusions in thalassemia major [11-13].
Seeing the varied uses and it nutrition in the present paper Bio chemical Studies of Wheat was done in which the analysis of Qualitative and Quantitative phytochemical was carried out

2. Material and Methods

Sample Cullection

The whole plant of *Triticum aestivum* was collected in the month of 21 March and authenticated at Devendra Nager Panna, Madhya Pradesh.

Preparation of Sample

Seeds were cleaned manually and powder was made and kept in air light container for analysis.

Preparation of Extracts for Qualitative Analysis

25 g of wheat seed powder was dissolved separately in 100 ml of commercially available pure ethanol. The solution was kept at room temperature for seven days to allow the extraction of compounds from seeds. The solution for each weed variety was stirred after every 24 h using sterile glass rod. After seven days, the solution was filtered through Whatman filter paper no. 1 The solvent was evaporated and a black sticky substance was obtained that was stored in the refrigerator and suspended in 10% dimethyl sulfoxide prior to use.

Phytochemical analysis (Qualitative test): [14-17]

Different phytochemicals present in the plants was qualitatively analysed. The ethanol and aqueous extract were prepared and the different chemical compounds was detected.

Tannins

One ml of water and 1-2 drops of ferricchloride solution was added in 0.5 ml of extracted solution. And color was observed.

Saponin

Added a drops of sodium bi carbonate solution in aqueous extract of seed powder shacked the mixture vigorously and kept aside for three minutes.

Flavonoids

To 0.5 ml of alcoholic extract added 5 to 10 drops of dil hydrochloric acid followed by a small piece of magnesium and observed.

Steroids

Two ml of acetic anhydride was added to 0.5 g of ethanolic extract followed by adding 2 ml H_2S0_4 and observation was made.

Terpenoids

Five ml of extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer.

Cardiac glycosides

5 ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1 ml of concentrated sulphuric acid.

Alkaloids

Added few drops of Mayer's reagents in the extract and observed.

Carbohydrate.

(a) By Anthrone solution

2 ml of anthrone solution was added to 0.5 ml of aqueous extract and observed

(b) By Fehling's solution

To 2 ml of aqueous extract added 1 ml of mixture of equal parts of fehling's solution "A" and fehling" solution "B" and boiled the contents of the test tube for few minutes and observed.

(c) By Molish reagent

In a test tube containing 2 ml of aqueous extract added 2 drops of a freshly prepared 20% alcoholic solution of naphthol and mixed 2 ml conc. Sulphuric acid so as to from a layer below the mixture.

Proteins.

To 1 ml of hot aqueous extract of the drug added 5 to 8 drops 10% w/v sodium hydroxide solution followed by 1 or 2 drops of 3% w/v copper sulphate solution.

Quantitative Phytochemical Analysis

Preparation of fat free sample:

Two g of the sample was defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 hours and alkaloid, saponin, flavonoids, carbohydrates, and cellulose were determined quantitatively by using standard methods [14 - 17].

Preparation of test Solution for Chromatography.

2 gram of finely grounded seeds were taken and refluxed it with petroleum, Then one gram powder was extracted with 10 ml ethanol at 60 °C for 5 minutes. The obtained solution was used directly for chromatography.

Stationary Phase: The pre-coated plates with silica gel 60F 254 of 0.2 mm thickness

Mobile Phase. *Tolune:* Ethyl Acetate (9:1)

Spray reagent: 5% Methanolic H_2SO_4

Results & Discussion

The results are tabulated in the table below i.e. table -1

Table 1: Qualitative analysis of phytochemical

S. No	Constituents	Observation	Standard Value	Result
1	Tannin	Light Yellow color Present	Green color	Negative
2	Saponin	Foam no appear after 10 minute	Foam appear	Negative
3	Flavonoide	Disappear yellow color	Colorless	Positive
4	Steroid	Brown color is produced	Green &Blue	Negative
5	Terpenoid	Reddish Brown Ring appear	Reddish Brown	Positive
6	Cardiac glycosides	Violet Ring appear	Violet Ring	Positive
7	Alkaloid	White color appear	Yellow	Negative
8	Carbohydrate			
(a)	Anthrone test	Blue color appearing	Blue color	Positive
(B)	Fehling test	Brick color appear	Brick color	Positive
(c)	Molish test	On adding excess of alkali red violet color disappear	red violet disappear	Positive
9	Proteins	Violet red color appear	Violet color	Positive
(a)	Million's test	Brick red color appear		

The Quantitative analysis of the Phytochemicals present in seed sample is given in table 2, 3, 4

Table 2: Quantitative analysis of Alkaloid

S.no	Sample weight	Initial Weight of Petridish	Final Weight of Petridish	Difference
1	5.0 g	30.32235 g	30.93078 g	0.60843 g

Alkaloid

= (Final Weight-Initial weight) x 100/Sample Weight
= 0.60843x 100/5
= 12.17%

Table 3: Quantitative analysis of Saponin

S.no	Sample weight	Initial Weight of Petridish	Final Weight of Petridish	Difference
1	20 g	30.46692 g	31.02357 g	0.56878 g

Saponin

= (Final Weight-Initial weight) x 100/Sample Weight
= 0.56878x 100/20
= 2.85%

Table 4: Quantitative analysis of Flavonoid

S.no	Sample weight	Initial Weight of Petridish	Final Weight of Petridish	Difference
1	10 g	44.12430 g	44.88200 g	0.7577 g

Flavonoid

= (Final Weight-Initial weight) x 100/Sample Weight
= 0.7577x 100/10
= 7.57%

The standard and sample table for carbohydrate is given in table 5 and 6 and standard and the sample table for cellulose is given in table 7 and 8.
The calibration curve for carbohydrate and cellulose is given in fig-1 and fig-2.

Table 5: (Standard table for carbohydrate)

S. N	Sample iD	Type Ex	Conc	WL630.0	Wgt. Factor
1	Std 1	Standard	0.200	0.278	1.000
2	Std 2	Standard	0.400	0.361	1.000
3	Std 3	Standard	0.600	0.532	1.000
4	Std 4	Standard	0.800	0.625	1.000
5	Std 5	Standard	1.000	0.821	1.000

Table 6: (Sample table for carbohydrate)

S. N	Sample iD	Type Ex	Conc	WL630.0
1	Blk	Unknown	-0.248	-0.048
2	Wheat 1	Unknown	3.964	2.792
3	Wheat 2	Unknown	3.949	2.782

Table 7: (Standard table for cellulose)

S. N	Sample iD	Type Ex	Conc	WL630.0	Wgt. Factor
1	Std 1	Standard	0.400	0.178	1.000
2	Std 2	Standard	0.800	0.275	1.000
3	Std 3	Standard	1.200	0.393	1.000

Table 8: (Sample table for cellulose)

S. N	Sample iD	Type Ex	Conc	WL630.0
1	Blk	Unknown	0.300	0.147
2	Wheat 1	Unknown	0.447	0.187
3	Wheat 2	Unknown	0.462	0.191

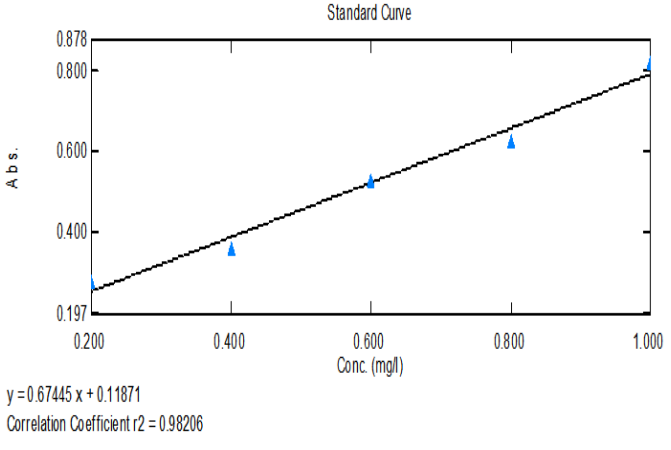


Fig 1: Calibration curve for carbohydrate

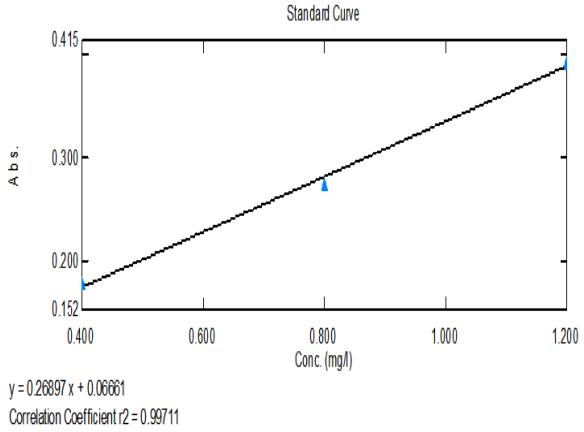


Fig 2: Calibration curve for cellulose

HPTLC

The HPTLC was performed and R_f values were recorded at 366 nm and 254 nm after derivatization (Table 9, 10). And in White light, BD, and White-R AD in (Table 11, 12), fig-3, 4, 5, 6.

HPTLC (R_f value and color of Band)

Table 9: R_f value of 366nm (after derivatization)

Ethanol	Color	Methanol	Color
0.48	Sky blue	0.48	Sky blue
0.075	Sky blue	0.75	Sky blue

Table 10: R_f value of 254nm (after derivatization)

Ethanol	Color	Methanol	Color
0.16	Faint brown	0.16	Faint brown
0.30	Light pink	0.30	Light pink
0.37	Faint brown	0.37	Faint brown
		0.69	Light blue
0.85	light brown	0.85	light brown
0.88	Dark brown	0.88	light brown

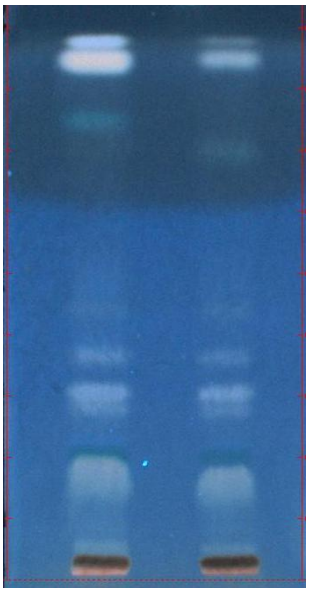
Table 11: R_f value white-R (before derivatization)

Ethanol	Color	Methanol	Color
0.16	Faint White	0.16	Faint white
0.30	Faint blue	0.30	Faint blue
0.36	Faint White	0.36	Faint white
		0.69	Faint White
0.75	Light blue	0.75	Light blue
0.84	White	0.84	White

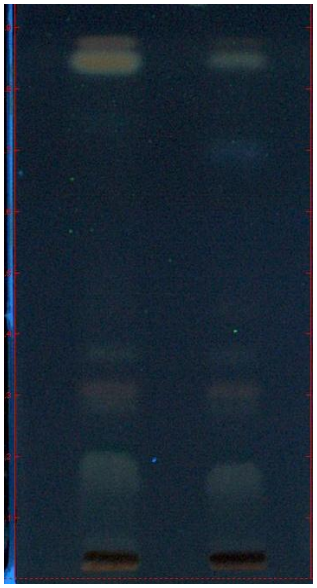
Table 12: R_f value of white R (after derivatization)

Ethanol	Color	Methanol	Color
0.16	Light brown	0.16	Light brown
0.31	Light brown	0.31	Light brown
0.36	Light brown	0.36	Light brown
0.84	Dark brown	0.85	light brown
0.88	Light blue	0.88	light brown

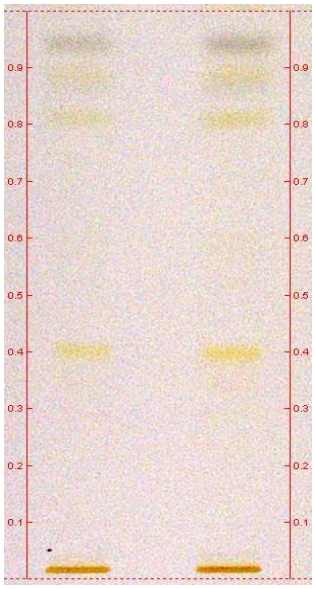
HPTLC Chromatogram



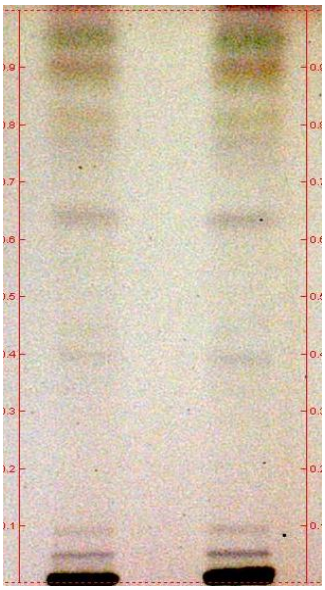
366nm AD
Fig – 3



254nm AD
Fig – 4



White- R BD
Fig – 5



White- R AD
Fig - 6

HPTLC Chromatogram

Qualitative analysis of wheat clearly reveals the presence of Flavonoid, Terpinoid, Cardiac glycoside, Carotenoids, Glycosides, Carbohydrate, and Protein out of which Carbohydrate gave positive result with Fehling's, Molish reagent and Anthrone reagent, which show that it is present in good amount. While protein was the second largest phytochemical.

All other phytochemical like Flavonoid, Terpenoid, Cardiac glycosides, Carotenoid, Glycosides, are present in very small amount and Tannin, Saponin, Steroid, Alkaloid, Anthroquinone, were totally absent. HPTLC was done at 366 nm, 254 nm and in visible light after derivatization and HPTLC chromatogram shows many spots in these chromatogram which shows the presence of various biomolecules.

3. Conclusion

Wheat (*Triticum aestivum* L.) plays a key role in traditional health care system for human and animals. Wheat is an important component of the human diet, and the effect on human health of bioactive compounds present in wheat has becoming a fascinating and important subject of study. The beneficial effects of wheat are still under debate because of the great number of potential health-promoting components present in the grains and the complexity of studying their biological effects. In Present study qualitative and quantitative phytochemical analysis of wheat extract was made. Various bioactive compounds like alkaloids, saponins, glycosides, terpenoids, steroids, flavonoids and tannins were detected. The presence of such compounds in plants might be responsible for their curative effect as they are the secondary metabolites and they act as chemical messenger or phytochemical regulator, which can inhibit the cell cycle. HPTLC studies of alcoholic extracts were carried out and HPTLC chromatogram shows many colored spots which indicate the presence of biomolecules the findings of the study reveals a strong hope for development of more chemotherapeutic agents.

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