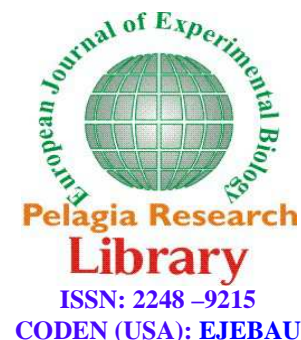




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Laboratory investigation on the role of *Triticum aestivum* (Linn.) in ameliorating various diseased conditions

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ABSTRACT

Triticum aestivum has been traditionally known to have several medicinal properties to treat various disorders. It has been used as a general purpose health tonic for several years. It is used to treat a number of conditions including the thalassaemia, hypolipidemic, hypoglycemic, ulcers, cleansing, fissures, injuries, cuts and wounds. In folk medicine, practitioners used wheatgrass for the treatment of cystitis, gout, rheumatic pain, chronic skin disorders and constipation. The present study was carried out to investigate total phenolic, total flavonoid content and estimation of quercetin by high performance liquid chromatography in methanolic extract of *Triticum aestivum* ninth day grass. Total phenolic content was determined by using the folin ciocalteu assay. The content of total flavonoids was also measured spectrophotometrically by using aluminum chloride colorimetric assay. Total phenolic content and total flavonoid content analysis was performed using methanol as solvent, gallic acid and rutin were used for standard curve respectively. High performance liquid chromatography was used for the estimation of quercetin in the extract. It is a powerful analytical technique because of its reliability, simplicity, reproducibility and speed. The purpose of the work is to investigate the chemical constituents responsible for the role of wheatgrass which has been used in traditional medicine to improve healthcare.

Key words: *Triticum aestivum*, total phenolic content, total flavonoid content, quercetin, high performance liquid chromatography.

INTRODUCTION

Phenolics are omnipresent secondary metabolites in plants which are considered to be large group of biologically active ingredients from simple phenol molecules to polymeric structures. There are two basic groups of phenolics simple phenols and polyphenols, classified on the basis of phenol subunit. Simple phenols comprises of phenolic acids or phenols with carboxyl group having the specificity of their function. Polyphenols comprises of at least two phenol rings. The most intensive work performed in recent years of this group is Flavonoids. More than 4000 flavonoids have been identified in different higher and lower plant species. [1]

The flavonoid pigments are usually yellow in color and are widely distributed in nature. They are difficult to separate and purify by crystallization due to their similar solubility relationships and their tendency to form mixed crystals. [2] Hence flavonoids are polyphenolic secondary plant metabolites which impart color and flavor to the plant foods. Many of these compounds play a protective role in the plants and are biochemically active. Flavonoids

have been regarded both for nutritive purpose as vitamins and also as anticarcinogens. [3] A number of its action makes it a potential anti cancer agent, including cell cycle regulation, interaction with type II Estrogen binding sites and tyrosine kinase inhibition. Bioflavonoids are a group of polyphenolic substances which are present in most plants, concentrated in the seeds, fruit skin, peel, bark and flowers. [4] Quercetin is termed as the aglycone or sugarless form of rutin and is the major bioflavonoid in the human diet. [5] It is a plant pigment found in many foods such as onions, apples, berries, tea, grapes and red wine. It is not a nutrient, but is classified as a flavonoid. Like many other plant chemicals, it is sold as a supplement. Oral quercetin is relatively well absorbed and it is metabolized mainly to isorhamnetin, tamarixetin and kaempferol. [6]

Investigations of the distribution of the major flavonoids in plant foods have already been reported. High performance liquid chromatography has been especially widely used for separation and determination of flavonoids in a variety of foods. [7–15] The aim of the present work is to investigate the chemical constituents responsible for curing various conditions and thereby increasing the potency and the activity of the wheatgrass by specificity.

MATERIALS AND METHODS

Collection and identification of the plant material

The whole plant of *Triticum aestivum* was collected on the ninth day during its jointing stage. It was authenticated at Safia college of Science Bhopal, Madhya Pradesh. The herbarium of the plant was prepared and the voucher specimen 236/BOT/SAFIA/2011 was obtained.

Preparation of Triticum aestivum powder

The Wheat grass seeds for the research were purchased from Breeder Seed Production Unit Field crops, Department of Plant Breeding and Genetics, Jawahar Lal Nehru Krishi Vishwavidyalaya, Krishi nagar, Jabalpur, M.P. and the release order number was obtained. The ninth day grass of *Triticum aestivum* was cultivated and collected. It was chopped with the help of knife, dried in shade and then powdered with a mechanical grinder. The powder was passed through sieve no. 40 and stored in a labeled air tight container for further studies.

Extraction by Maceration

Maceration process involves separation of medicinally active portions of the crude drugs. It is based on the immersion of the crude drugs in the bulk of solvent or menstrum. Solid drug material is taken in a stoppered container with about 750ml of the menstrum and allowed to stand for at least three to seven days in a warm place with frequent shaking. The mixture of crude drug containing solvent is filtered until most of the liquid drains off. The filtrate and the washing are then combined to produce 1000ml of the solution. Then the extract was formed by drying it in a hot air oven for 30 minutes at 40°C. The prepared extract is then collected and stored in a dark colored bottle for further studies. [16, 17]

Total phenolic content

The amount of total phenolic content in extract was determined with the folin ciocalteu reagent. Gallic acid was used as a standard and the total phenolic content was expressed as mg/gm Gallic acid equivalent (GAE). Concentrations of 0.01 to 0.1mg/ml of Gallic acid were prepared in methanol. Concentration 1mg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced into test and mixed with 2.5ml of a 10 fold dilute folin ciocalteu reagent and 2ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was read at 760nm spectrophotometrically. All determination was performed in triplicate. The folin ciocalteu reagent is sensitive to reducing compounds including polyphenols, there by producing a blue color upon reaction. This blue color is measured spectrophototmetrically. Thus total phenolic content can be determined. [18]

Total flavonoid content

For total flavonoids content different concentration of plant extract (1mg/ml) and different concentration of rutin as a standard were taken. 0.5ml of sample solution was taken and 2.5ml of distilled water and 0.15ml of 5% NaNO₂ was added. After 6min, 75µl of 10% AlCl₃ was added. After another 5min, 0.5ml of 1 M NaOH was added to the mixture. Immediately, the absorbance of the mixture was determined at 510nm versus prepared water blank. [19]

Chromatographic profile*Thin layer Chromatography*

Prepared TLC plate by using silica gel G 60 and activated in hot air oven at 100°C for 30min is used for the study. Taken TLC chamber, washed with solvent then saturated with solvent (Ethyl acetate: Methanol: Water 25: 4.1: 3.3 v/v) for 30min. Applied small spot of the diluted extract on a prepared TLC plate and developed the plate to a desired distance from the line of application. Dried the plate in air and observed under UV light and Iodine vapours, the spots were clearly visible. The chromatogram showed a spot at corresponding R_f value to that of Quercetin. [20, 21]

*High performance liquid chromatography (Estimation of Quercetin in methanolic fraction by HPLC)**Chromatographic system:*

High Performance Liquid Chromatographic system (YL-Clarity 9100) equipped with universal injector, injection volume 20µl, PDA (UV-Vis) detector. A Promosil C₁₈ column (250 × 4.6mm) with particle size 5µm forms the stationary phase.

Table no. 1: Selection of separation variable

Column	
Variable	Condition
Dimension	250mm x 4.60mm
Particle Size	5µm
Bonded Phase	Lichrosphere (C18)
Mobile Phase	
Variable	Condition
Solvent A- Orthophosphoric acid	0.5% v/v
Solvent B ratio - Water:MeOH	40:60 v/v
Flow rate	1.0ml/min
Temperature	300C
Sample Size	20µl
Detection wavelength	370nm

Standard preparation

Standard quercetin (10mg) was transferred to 10ml volumetric flask and dissolved in 5ml methanol and the solution was made up to 10ml with the same solvent (1mg/ml).

Sample preparation

The sample of plant material was powdered and sieved through a 0.315mm sieve. An accurately weighed mass (300–500mg leaves) was first defatted by pre-extraction with chloroform to remove waxes (after filtration, the chloroform extract was discarded) and refluxed for 1h with 30ml of 95% (v/v) methanol. After filtration, the sample was extracted twice with 20ml of methanol for 10min. The combined extracts were diluted with methanol to 100ml and filtered through a PTFE syringe filter (13mm, 2µm, Whatman, UK). The filtrate was directly injected (20µl) into the HPLC system. Determinations were performed after three separate extractions of each sample and each extract was injected in triplicate.

Procedure

Set the instrument as per the chromatographic condition prescribed above. By means of suitable syringe inject 20µl of standard solution. Record the chromatograms and calculate the area of chromatogram. Calculate the percentage of quercetin content from the peak areas. [22]

RESULTS AND DISCUSSION

The plant of *Triticum aestivum* which belongs to the family poaceae is an indigenous herb which has been chosen for this study. The ample availability of information on this plant facilitates the study on it since ages various parts of this plant are being used for their medicinal use. The attempt is made to estimate the total phenolic content, total flavonoid content, thin layer chromatography and high performance liquid chromatography for quercetin from the plant of *Triticum aestivum* and to develop a method for the estimation of quercetin content in wheatgrass extract. Due to its diversity in species, time of collection it remains a drug of challenge for its efficacy and potential in treating many harmful diseases. The result indicates that the plant has much potential if carefully and cautiously

used and could be candidate for other pharmacological activity. This work will surely help to increase the bioactivity of wheatgrass by specificity.

Total phenolic content

Total phenolic content analysis was performed in methanolic extract of *Triticum aestivum* and Gallic acid was used for standard curve. The standard curve of gallic acid is shown in graph no.01 and table no. 02. The total phenolic content analysis of extract is shown in table no. 03.

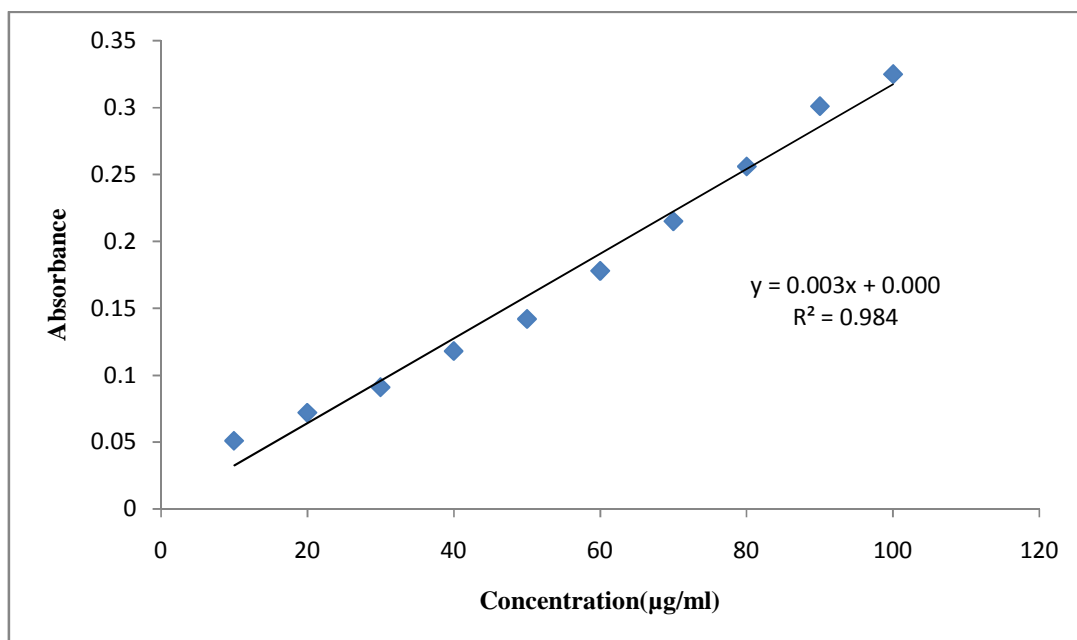
Table no. 2: Concentration and absorbance of standard Gallic acid

S. No.	Conc. (µg/ml)	Absorbance
1	10	0.051
2	20	0.072
3	30	0.091
4	40	0.118
5	50	0.142
6	60	0.178
7	70	0.215
8	80	0.256
9	90	0.301
10	100	0.325

Table no. 3: Observation of extract for total phenolic content

S. No.	Concentration (mg/ml)	Abs.	Gallic acid equivalent (µg/mg)
1	1 mg/ml	1.412	440.96
2	1 mg/ml	1.523	475.65
3	1mg/ml	1.356	423.46
		Mean	446.69
		s.d.	26.56

Graph no. 1: Standard curve of Gallic acid



Total flavonoid content

Total flavonoid content analysis was performed in methanolic extract of *Triticum aestivum* and Rutin was used for standard curve. The Standard curve of rutin is shown in graph no. 02 and table no. 04. The analysis of methanolic extract is shown in table no. 05.

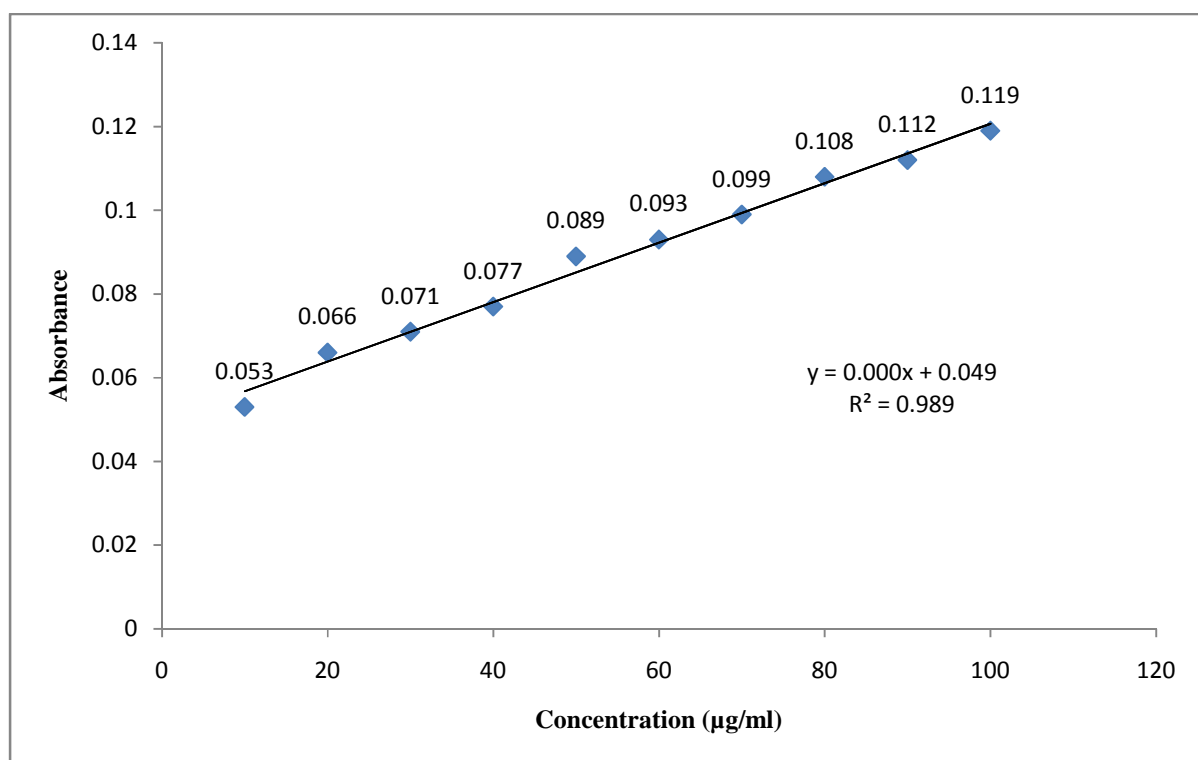
Table no. 4: Concentration and absorbance of standard Rutin for total flavonoid content

S. No.	Conc. (µg/ml)	Absorbance
1	10	0.053
2	20	0.066
3	30	0.071
4	40	0.077
5	50	0.089
6	60	0.093
7	70	0.099
8	80	0.108
9	90	0.112
10	100	0.119

Table no. 5: Observation of extract for total flavonoid content

S. No.	Concentration(mg/ml)	Abs.	Rutin equivalent(µg/mg)
1	1 mg/ml	0.123	104.72
2	1 mg/ml	0.145	136.14
3	1mg/ml	0.151	144.71
		mean	128.53
		s.d.	21.05

Graph no. 2: Standard curve of Rutin

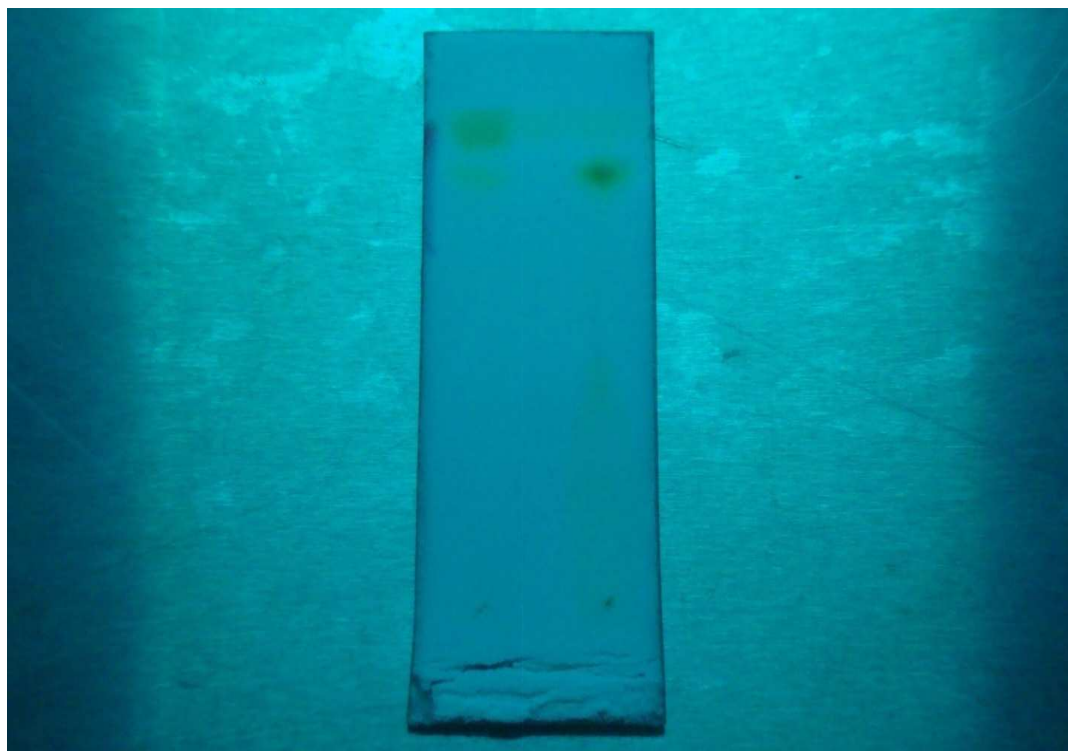


Chromatographic profile

Thin layer Chromatography

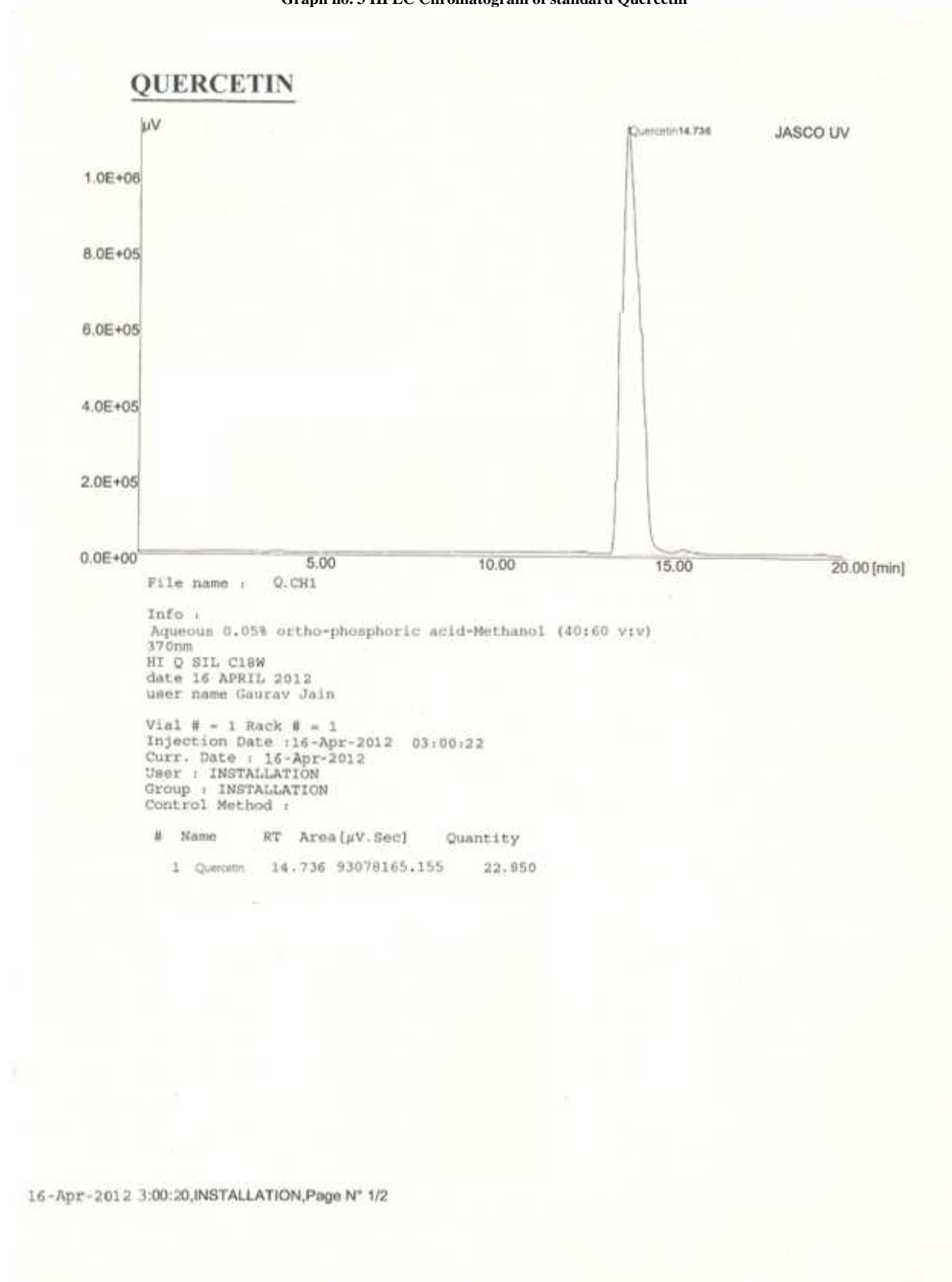
This study showed the presence of one spot of the methanol extract at R_f value 0.67 and one spot of standard quercetin at R_f value 0.51 in the solvent system of Ethyl acetate: Methanol: Water (25: 4.1: 3.3). Resolved component was visualized under UV light and Iodine vapors as detecting agent. The TLC plate is shown in figure no. 1.

Figure no. 1 TLC of methanolic extract and standard Quercetin

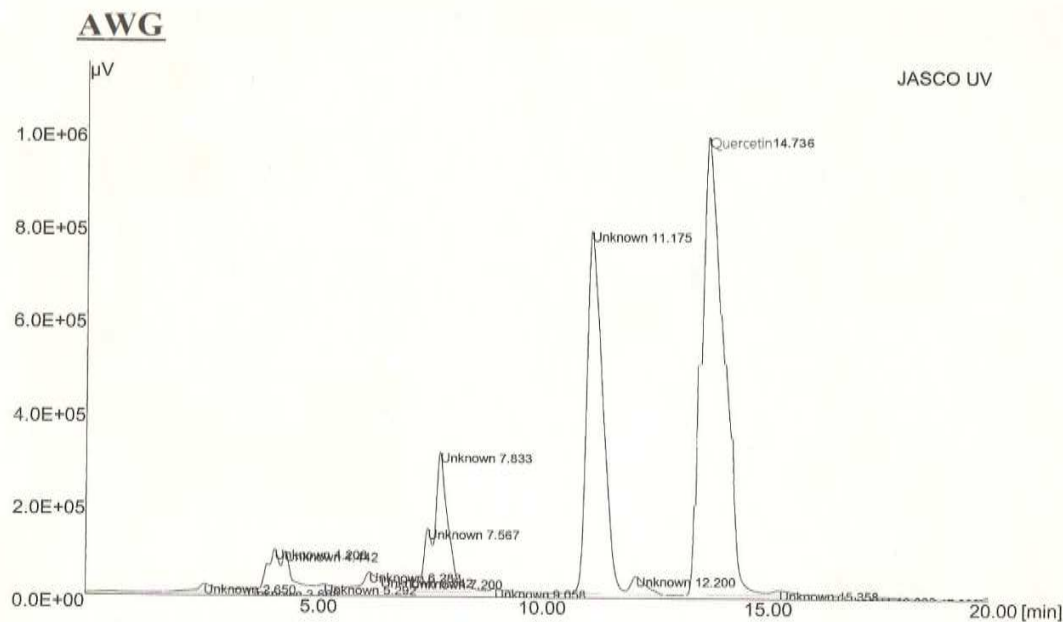
**High performance liquid Chromatography**

The retention time and area of peak of standard quercetin were found to be 14.736 and 93078615.155 respectively, which it is represented in graph no. 03. The retention time and area of peak of isolated quercetin from methanolic extract of *Triticum aestivum* were found to be 14.736 and 53170901.846 respectively, which it is represented in graph no. 04.

Graph no. 3 HPLC Chromatogram of standard Quercetin



Graph no. 4 HPLC Chromatogram of isolated Quercetin



Info :
 Aqueous 0.05% ortho-phosphoric acid-Methanol (40:60 v:v)
 370nm
 HI Q SIL C18W
 date 16 APRIL 2012
 user name Gaurav Jain

Vial # = 1 Rack # = 1
 Injection Date :16-Apr-2012 13:00:52
 Curr. Date : 16-Apr-2012
 User : INSTALLATION
 Group : INSTALLATION
 Control Method :

#	Name	RT	Area [μV.Sec]	Quantity
1		2.650	521039.573	0.000
2		3.608	226734.818	0.000
3		4.208	1632928.336	0.000
4		4.442	1463460.645	0.000
5		5.292	407932.927	0.000
6		6.283	1025981.800	0.000
7		6.542	786648.218	0.000
8		7.200	501223.418	0.000
9		7.567	1563153.764	0.000
10		7.833	5399161.773	0.000
11		9.058	104365.600	0.000
12		11.175	19096864.800	0.000
13		12.200	908486.195	0.000
14	Quercetin	14.736	53170901.846	22.850
15		15.358	332378.756	0.000
16		16.633	25623.700	0.000
17		17.608	47635.683	0.000

Total Area of Peak = 87214521.853 [μV.Sec]

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CONCLUSION

Many efforts have been devoted for isolating many biologically active compounds from wheatgrass. In the present work phenolic and flavonoid compounds were estimated and especially quercetin in wheatgrass and their role in laying down standardization and pharmacopoeial parameters to make it useful for further pharmacological evaluation. Chromatographic studies showed the presence of quercetin which is a good antioxidant agent. It has proved to be a potential drug having high medicinal values. This work is a step forward in determining the role of *Triticum aestivum* being used traditionally for the ailment in various diseased conditions and to develop an accurate, specific, repeatable and robust method for the estimation of Quercetin content in the wheatgrass extract.

Acknowledgement

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