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DETERMINATION OF ANTIOXIDANT ACTIVITY IN ALUMINIUM INDUCED OXIDATIVE STRESS IN CHICKEN EMBRYO USING GABA FROM GERMINATED BROWN RICE AND GC-MS ANALYSIS OF GBR

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ABSTRACT

Brown rice is scientifically known as *Oriza sataiva*. It is a pigmented rice and it has many bioactive compounds such as antioxidant, anti inflammatory, anti diabetic, anti microbial, anti malaria. It has anti oxidant property. In this work we have used germinated brown rice, because after germination it shows the rich contents of antioxidants like Vitamin E, Proteins, healthy fats and fiber. In this study, we are evaluating and comparing the antioxidant property of GBR and non GBR through Phytochemical analysis, GCMS analysis, Estimation of Antioxidant activity by the estimation of DPPH scavenging, TPC, TFC, SOD and H_2O_2 scavenging. As GBR Contains more amount of alkaloids & flavanoids than non-GBR. Since intake of excess Aluminium creates cell damage and produces stress. Since GBR has high GABA(Stress Inhibitory Neurotransmitter) content, this project is based on the antioxidant activity of GBR against Aluminium induced stress using chick eggs.

Keywords: Brown rice, Antioxidant activity, Alkaloids and Flavonoids, Phytochemical analysis, GBR, BR, Aluminium toxicity, Chicken embryo.

INTRODUCTION

Rice is a major cereal crop consumed as a staple food by over half of the world's population. The scientific name of rice is *oryza sativa* and *oryzaglaberrima* where*oryza sativa* was originated in Asia and *oryzaglaberrima* in is cultivated in Africa. Rice are of different types depending upon their colour, size, shape etc. But two main types are brown rice and white rice. Brown rice is a whole grain in which inedible outer

layer (husk) is removed where as in the white rice husk, bran and Germ are removed (2).Brown rice is not consumed because it requires longer cooking time, has an undesirable color, and a firm texture. However, in recent years the nutritional significance of brown rice has been recognized and its use is being encouraged(3).Antioxidants are compounds that slow or prevent the oxidation of other molecules, which produce free radicals that start chain reactions and damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by oxidizing themselves(3).brown rice is associated with a wide spectrum of nutrigenomic implications such as anti-diabetic, anti-cholesterol, cardioprotective and antioxidant.

GBR

Germinated brown rice is considered healthier than white rice, as it is not only richer in the basic nutritional components such as vitamins, minerals, dietary fibers, and essential amino acids, but also contains more bioactive components, such as ferulic acid, γ-oryzanol, and gamma aminobutyric acid. The unprocessed brown rice is germinated to upgrade the flavour and texture and also to increase the level of nutrient is called germinated brown rice or GBR .Germinated brown rice (GBR) has the highest antioxidant activity compared to white rice and brown rice.

ANTI-OXIDANT

Antioxidants plays an important role in food preservation by inhibiting oxidation processes and contributing to health promotion rendered by many dietary supplements, neutraceuticals and functional food ingredients. Antioxidant activity can be monitored by a variety of assays with different mechanisms, including hydrogen atom transfer (HAT), single electron transfer (ET), reducing power, and metal chelation, among others.

PHYTOCHEMICAL ANALYSIS

Phytochemicals are the chemicals that present naturally in plants. Now- a-days these phytochemicals become more popular due to their countless medicinal uses. Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc. unlike pharmaceutical chemicals these phytochemicals do not have any side effects.The effectiveness of antioxidants is generally influenced by a number of factors, including their structural features, concentration, temperature, type of oxidation substrate and physical state of the system as well as presence of pro-oxidants and synergists.

GC-MS

Gas Chromatography- Mass Spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within the test sample.gas chromatography coupled with mass spectrometry (GC--MS) has become an established method for investigating organic materials in art and archaeological objects.GC-MS allows the measurement of various metabolites . This comprises a number of volatiles such as ketones, aldehydes, alcohols, heterocyclic compounds, isocyanates, isothiocyanates, sulfides, lipids, and hydrocarbons up to 12 carbons, which all can be directly measured. Like liquid chromatography – Mass spectrometer, it allows analysis and detection even of tiny amounts of a substance.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL IDENTIFICATION:

Fresh samples of brown rice (*Oryza sativa L*) were collected from Nenmara province, Palakkad and the cleaned sample was soaked for 2 days for germination. The germinated rice sample was powdered and stored at room temperature till used for further studies.

PREPARATION OF THE EXTRACT

About 5g of air-dried germinated brown rice sample was extracted by simple filteration with ethanol, methanol, chloroform, aqueous and petroleum ether separately. Each time before extracting with the next solvent, the powdered material was dried. Then, these extract were used for further analysis.

PHYTOCHEMICAL ANALYSIS

To identify the phytochemical present in the ethanol, methanol, chloroform, aqueous and petroleum ether extract of Germinated brown rice (*Oryza sativa*), Qualitative analysis were carried out according to the phytochemical screening methods.

Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography-mass spectrometry is an analytical technique that combine the features of gas chromatography and mass spectrometry to identify different substances within a test sample. It is a separation technique of choice for smaller and volatile molecules such as benzenes, alcohols and aromatics, and simple molecules such as steroids, fatty acids and hormones. It is widely used for chemical analysis, and especially for drug and environmental contamination testing.

GC-MS sample analysis method

The chemical composition of the *oryzasativa* samples extracted in aqu was analysed using GC-MS. The samples were filtered through 0.22 µm syringe filter prior to analysis. 1 µl each of filtered sample was analysed using GC-MS (QP-2010-Shimadzu) equipped with a Rxi-5Sil MS column of 30m in length, 0.25mm in diameter, and 0.25µm thickness. The GC-MS was employed with helium as carrier gas at a constant flow of 1ml/minute. The oven temperature was started at 80^0 and remained at this temperature for 4 minutes and, increasing to 280^0 at 5^0/min ramp rate. Injection port was adjusted at 260^0 and split less injection mode was used. EI mode was at 70 eV, while mass spectra were recorded in 50-500 amu range and ion source temperature was maintained at 200^0. The components of *Oryzasativa*samples were identified by comparing the retention time of chromatographic peaks using quadrupole detector with NIST library.

ANTIOXIDANT ACTIVITY OFGERMINATED BROWN RICE*(Oryza sativa)*

AIM:

To evaluate the antioxidant activity of *Oryza sativa L* on Aluminium induced oxidative stress in chicken embryo.

REQUIREMENTS:

- Chicken egg
- Syringe (Insulin injecting)
- Reagents and arrangements required for antioxidant assay.

PROCEDURE:

To evaluate the antioxidant activity of *Oryza sativa L*, chicken eggs were used, 15 healthy 5 day old country chicken egg were obtained from chicken farm, and the work was carriedout at laboratory. The eggs were maintained at controlled environmental conditions before and during the experiments . They were randomly divided into 5 groups(3 eggs in each group). Group I eggs were treated as control, group II eggs were injected with GBR sample, group III eggs were injected with aluminium chloride solution, group IV eggs were injected with GBR sample and aluminium chloride solution, group V eggs were injected with standard drug for 5-6 days. On the 7^{th} day of experiment eggs from each groups were dissected and it's embryos were collected. The embryo samples collected were used to estimate the Enzymatic and Non-enzymatic assay, DPPH assay. Then

the results are compared to know the antioxidant activity of Germinated brown rice against the metal induced oxidative stress.

ENZYMATIC ANTIOXIDANT

a)ESTIMATION OF SUPER OXIDE DISMUTASE

Superoxide anions were generated in samples that contained in 3.0ml, 0.02ml of the fruit extract (20mg), 0.2ml of EDTA, 0.1ml of NBT, 0.05of riboflavin and 2.64ml of phosphate buffer. The control tubes were also set up where DMSO was added instead of the plant extracts. All the tubes were vortexed and the initial optical density was measured at 560nm in a spectrophotometer. The tubes were illuminated using a fluorescent lamp for 30 minutes. The absorbance was measured again at 560nm. The difference in absorbance before and after illumination was indicative of superoxide anion scavenging activity.

b) ESTIMATION OF HYDROGEN PEROXIDASE

A solution of H2O2 (40mM) was prepared in phosphate buffer. Fruit extract at the concentration of 10mg /10µl were added to H2O2 solution (0.6ml) and the total volume was made up to 3ml. The absorbance of the reaction mixture was recorded at 230nm in a spectrophotometer. A blank solution containing phosphate buffer, without H2O2 was prepared. The extent of H2O2 scavenging of the plant extract was calculated.

NON ENZYMATIC ANTIOXIDANT

a)ESTIMATION OF FLAVONOIDS

Added 0.5 ml of the sample into a test tube containing 1.25ml of distilled water. Then added 0.075 ml of 5% sodium nitrite and allowed to stand for 5 minutes. Added 0.15ml of 10% aluminum chloride ,after 6 minutes 0.5ml of 1M NaOH was added and the mixture was diluted with another 0.75ml of distilled water. The absorbance of the mixture at 510nm was measured immediately. The flavonoid content was expressed as milligram catechin equivalents/g sample.

b) ESTIMATION OF PHENOL

Pipetted out different aliquotes(0.2-1 ml) of the standard into test tube. Made the volume upto 3 ml with water and added 0.5 ml Folin-Ciocalteau reagent. After 3 minutes, added 2 ml of 20% sodium carbonate to each test tubes. Mixed thoroughly , placed in a boiling water bath for exactly 1 minute. Cooled and measured the absorbance at 660 nm colorimetrically. Prepared calibration curve to different concentration of catechol.

ESTIMATION OF ANTIOXIDANT USING DPPH ASSAY

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2picryl-hydrazyl (DPPH) according to the previously reported method by (Shen et al., 2010). Briefly, an 0.1mM solution of DPPH in methanol was prepared and 1mL of this solution was added to 3 ml of the solution of all extracts in methanol at different concentration (50,100,200,400 &800µg/mL).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UVVIS spectrophotometer. Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity.

RESULTS AND DISCUSSION

PHYTOCHEMICAL SCREENING

Table: 1

SL.NO	COMPOUND	AQUEOUS	ETHANOL	METHANOL	CHLOROFORM	PETROLEUM ETHER
1.	Alkaloids	-	+	-	+	-
2.	Flavanoids	+	-	+	-	+
3.	Tannins	-	+	-	-	+
4.	Steroids	-	-	+	-	-

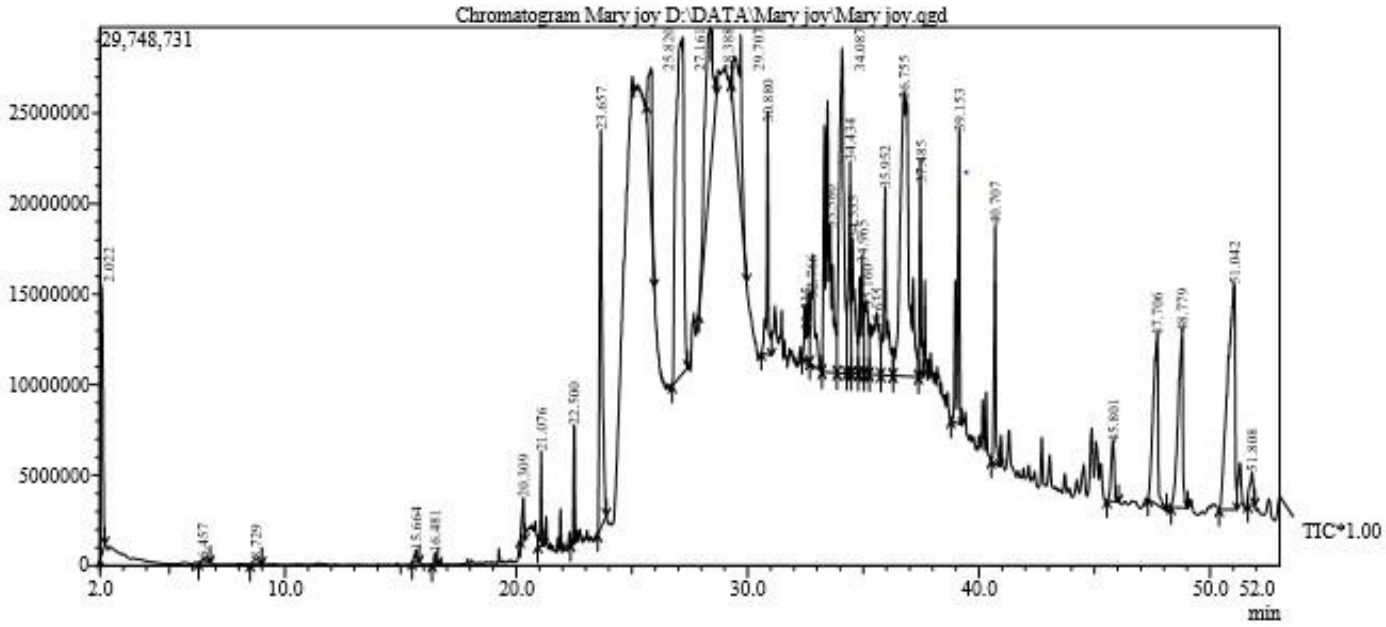
5.	Saponins	-	+	-	-	-
6.	Glycosides	+	+	-	-	-
7.	Phenols	+	+	++	-	-
8.	Carbohydrates	++	+	++	-	-
9.	Proteins and aminoacids	++	++	++	+	+
10.	Diterpenes	-	+	-	-	+

+ = Presence of Phytochemical, - = Absence of Phytochemical

Discussion:

Germinated brown rice represent a source of phytochemicals, such as amino acids, phenolics, dietary fibreand flavonoids. These are rich in almost all solvent extracts above especially in Ethanol, Methanol, and aqueous. The presence of alkaloids and diterpenes are minimum.

GC-MS ANALYSIS



MASS SPECTROMETRY OF GCMS ANALYSIS

GC-MS CHROMATOGRAM OF THE GERMINATED BROWN RICE – ETHANOL EXTRACT

Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Mark	Name
1	2.022	2.005	2.210	109029955	15231739	7.16		ETHANOL
2	6.457	6.260	6.770	307569312	12489670	1.82	MI	BENZOFURAN, 2,3-DIHYDRO-
3	8.729	8.495	9.000	486929134	13562748	2.11	MI	2-Methoxy-4-vinylphenol
4	15.664	15.495	15.800	527883473	1537546	3.20	MI	Dodecanoic acid
5	16.481	16.360	16.615	319414721	2126453	1.58	MI	1-NONADECENE
6	20.309	20.165	20.370	14434126	2366781	6.10	MI	Tetradecanoic acid
7	21.076	20.925	21.180	19169803	5225611	3.67	MI	1-Nonadecene
8	22.500	22.345	22.500	14796573	6569139	2.25	MI	9-Heptadecanone
9	23.657	23.520	23.895	179586423	21953719	14.30		HEXADECANOIC ACID, METHYL ESTER
10	25.820	25.630	25.960	79675683	7815793	10.19		HEXADECANOIC ACID
11	27.161	26.740	27.410	439531055	18491181	18.26		9-OCTADECENOIC ACID (Z)-, METHYL ESTER
12	28.388	27.890	28.660	217382852	7941251	27.37		E,E-3,13-Octadecadien-1-ol
13	29.707	29.285	29.960	142338873	8903067	15.99		Z-9-Pentadecenol
14	30.880	30.585	31.035	68129511	12654722	7.67		Ethanamine, 2,2'-oxybis[N,N-dimethyl-
15	32.535	32.360	32.685	35900523	1628949	22.04		ETHYL DOCOSANOATE
16	32.766	32.685	33.210	66181889	3928932	16.84	V	Heneicosane
17	33.580	33.210	33.860	232625425	7924892	29.35		9,12-OCTADECADIENOIC ACID (Z,Z)-, 2,3-DIHYDROXYPROPYL ESTER
18	34.087	33.860	34.285	248458971	17694836	4.81	V	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
19	34.434	34.285	34.485	75148130	11225974	5.05	V	HEXATRIACONTANE
20	34.535	34.485	34.785	70670572	7226921	9.78	V	1,2-Benzenedicarboxylic acid, diisooctyl ester
21	34.965	34.785	35.035	56146754	6153876	9.12	V	9-Octadecenoic acid (Z)-, tetradecyl ester
22	35.160	35.035	35.260	44715962	3900863	11.46	V	1,3-Benzenediol, 5-pentadecyl-
23	35.635	35.260	35.760	76852936	2877576	26.71	V	Hexadecane
24	35.952	35.760	36.285	91129424	9753809	9.34	V	Triacontane
25	36.755	36.285	37.385	412729696	15435531	3.07	V	9,12-OCTADECADIENOIC ACID (Z,Z)-, 2,3-DIHYDROXYPROPYL ESTER
26	37.485	37.385	37.585	56140527	11621215	6.99	V	HEXATRIACONTANE
27	39.153	38.785	39.285	113656369	15884252	12.36		2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-
28	40.707	40.535	40.910	68026677	12668315	11.36		Hexatriacontane
29	45.801	45.535	46.035	32615498	3246927	10.05		2H-1-BENZOPYRAN-6-OL, 3,4-DIHYDRO-2,7,8-TRIMETHYL-2-(4,8,12,16,20,24,28,32-OCTAMETHYL-3,7,11,15,
30	47.706	47.310	48.110	138656629	9254872	14.98		ERGOST-5-EN-3-OL, (3.BETA.,24R)-
31	48.779	48.310	49.035	137635682	9489605	14.50		Stigmasterol
32	51.042	50.385	51.160	277093698	12455156	7.80		ERGOST-5-EN-3-OL, (3.BETA.)-
33	51.808	51.625	51.930	19779712	1829446	10.81	MI	Cholest-4-en-3-one

Bioactive Compounds in ethanolic extractofGerminated Brown Rice.

ANTIOXIDANT ANALYSIS

❖ENZYMATIC ANTIOXIDANTS:

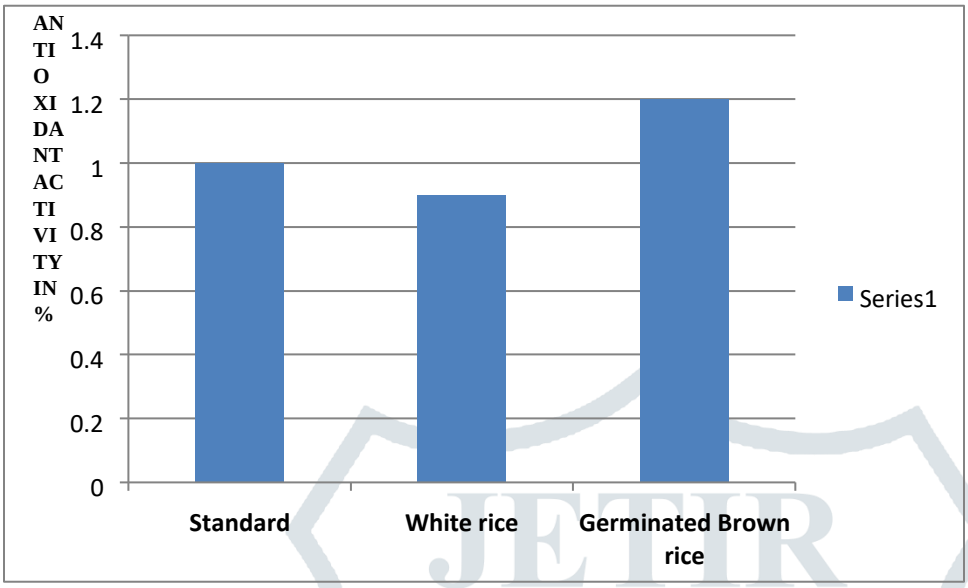
ESTIMATION OF SUPEROXIDE DISMUTASE:

OBSERVATION TABLE :

Table: 2

SL.NO	PARAMETERS	OBSERVATION
1	Standard	1.0
2	White rice	0.9
3	Germinated Brown rice	1.4

GRAPHICAL REPRESENTATION : 1



Discussion:

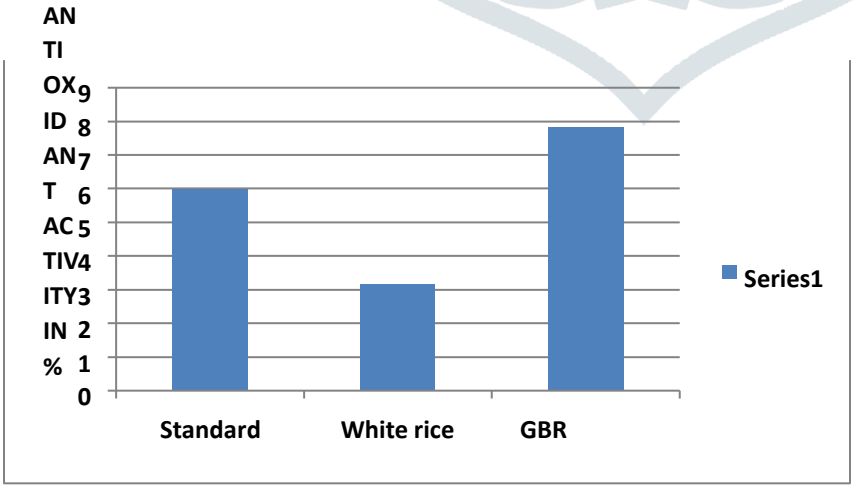
When compared to white rice GBR exhibits more antioxidant activity of Superoxide Dismutase.

ESTIMATION OF HYDROGEN PEROXIDASE:

OBSERVATION TABLE :

Table: 3

SL.NO	PARAMETERS	OBSERVATION (%)
1	Standard	5.98
2	White rice	3.16
3	GRAPHICAL REPRESENTATION : 2 Germinated Brown rice	7.83



Discussion:

When compared to white rice GBR exhibits more antioxidant activity of hydrogen peroxidase.

❖NONENZYMATIC ANTIOXIDANTS

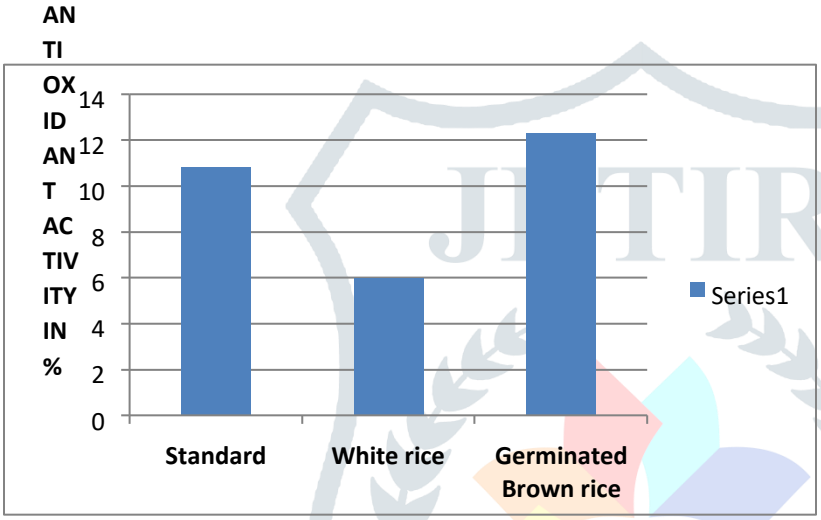
ESTIMATION OF PHENOLS:

OBSERVATION TABLE :

Table: 4

SL.NO	PARAMETERS	OBSERVATION (%)
1	Standard	8.03
2	White rice	5.98
3	Germinated Brown rice	12.3

GRAPHICAL REPRESENTATION : 3



Discussion:

When compared to white rice GBR exhibits more antioxidant activity of Phenol.

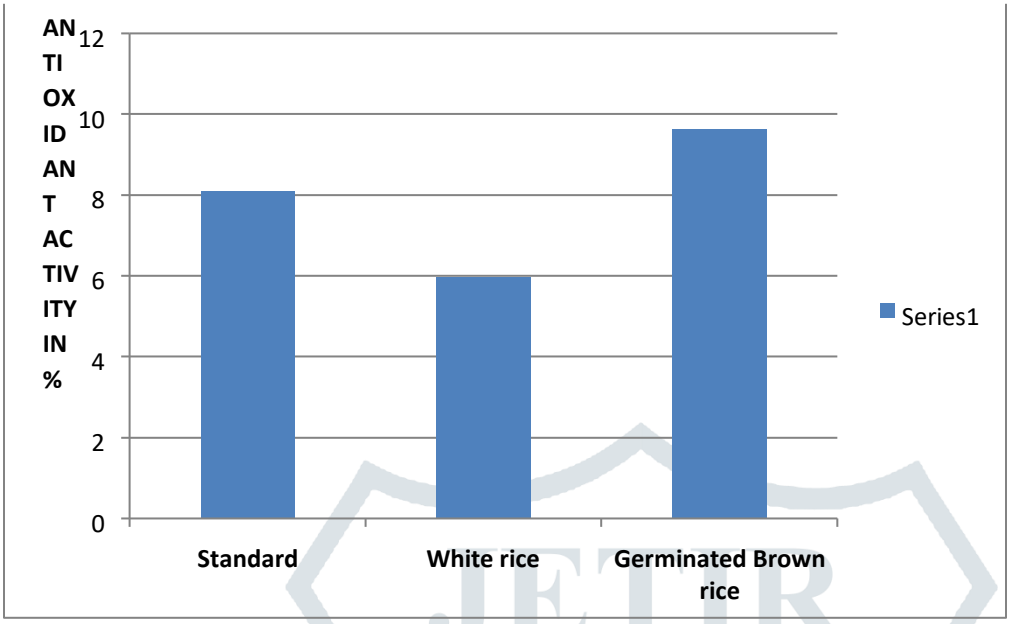
ESTIMATION OF FLAVANOIDS:

OBSERVATION TABLE :

Table: 5

SL.NO	PARAMETERS	OBSERVATION (%)
1	Standard	8.11
2	White rice	5.97
3	Germinated Brown rice	9.62

GRAPHICAL REPRESENTATION : 4



Discussion:

When compared to the white rice GBR exhibits more antioxidant activity or more flavanoid content.

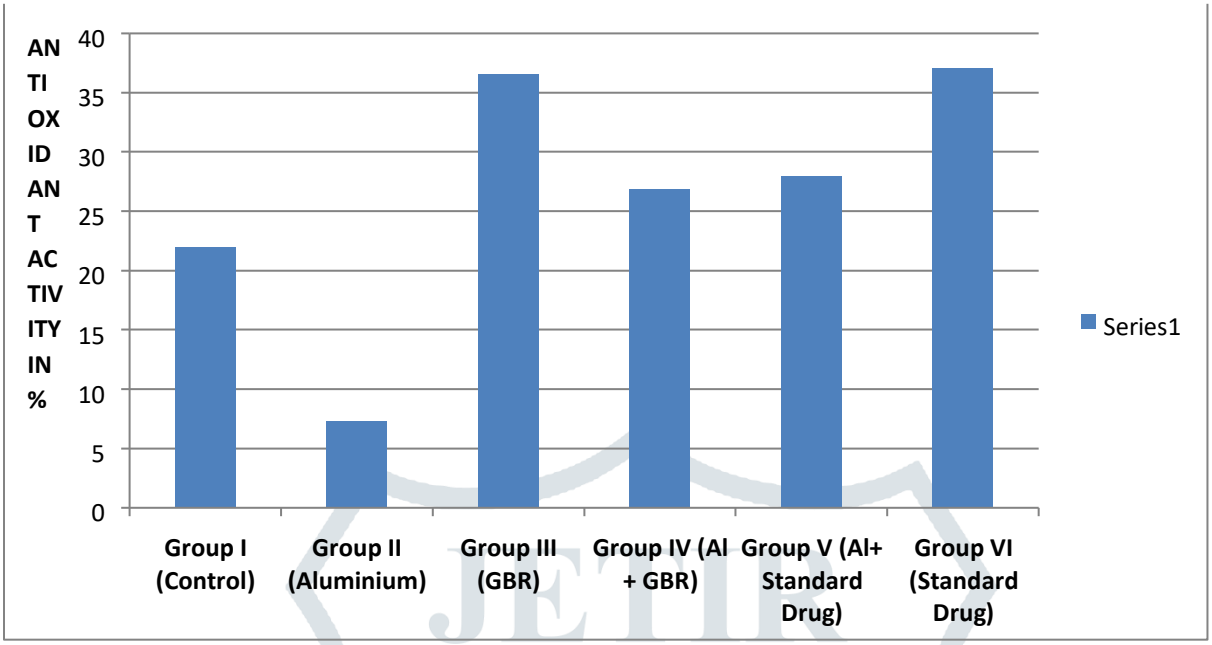
DPPH ANTIOXIDANT ASSAY:

OBSERVATION TABLE :

Table: 6

SL.NO	PARAMETERS	OBSERVATION (%)
1	Group I (Control)	21.95
2	Group II (Aluminium)	7.3
3	Group III (GBR)	36.58
4	Group IV (Al + GBR)	26.82
5	Group V (Al+ Standard Drug)	27.93
6	Group VI (Standard Drug)	37.1

GRAPHICAL REPRESENTATION : 5



Discussion:

Germinated Brown Rice given group shows the high antioxidant activity which almost equalises with the standard drug given group. The aluminium given group shows less antioxidant activity. In group IV, due to the presence of GABA in GBR the activity of antioxidant enzymes are increased and thus it reduces the oxidative stress caused by aluminium. The group IV and V are also compared.

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