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DETERMINATION OF ANTIOXIDANT PROPERTY OF PLANT EXTRACTS OF GERMINATED BROWN RICE (*ORYZA SATIVA L.*) AND COMPARISON WITH NON-GERMINATED BROWN RICE

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ABSTRACT

Brown rice (Oryza sativa L.), is a most important pigmented rice which is abundant in many bioactive compounds. Due to the presence of bioactive compounds, brown rice is known for different Properties such as antioxidant, anti-inflammatory, anti-diabetic, anti- microbial and anti-malarial properties. Antioxidant property is the capacity to hinder the oxidation of biological compounds, thereby reduce risk of degenerative diseases. Brown rice contain high antioxidant capacity than normal white rice varieties, since it is rich in polyphenols, flavonoids, vitamins, antho-cyanidins, etc. The germination process will enhance the amount of bio active compounds. There are large number of synthetic antioxidants, but taking plant-based food having antioxidant activity is always a good option. In this study, we are evaluating the antioxidant property of GBR through phytochemical analysis, GC-MS analysis, estimation of antioxidant activity by estimation of DPPH scavenging, TPC, TFC, SOD AND H2O2 scavenging.

KEYWORDS: Antioxidants, Degenerative disease, GBR, Bioactive compounds.

INTRODUCTION

Rice is a monocotyledonous annual grass plant of Poaceae family. The Main species of rice is Oryza sativa L., grown mainly in Asian countries[1] Rice (Oryza sativa L.) is one of popular staple food consumed by approximately 2.5 billion people.[2] According to the color of outer layer, the rice varieties classified in to brown rice, black rice, red rice and purple rice.[3] The brown rice is a whole grain and it consist of all parts of a rice kernel such as bran, germ endosperm.[4] Hence the brown rice is very nutritious since it contains all vital parts of rice grain so more amount of nutrients. Milling of brown rice yield white rice, which is tastier and have long shelf life. But, it losses the bran and germ portions by the process of milling. So that the nutritional quality of white rice is low compared to white rice.[3] The antioxidant property of brown rice is very high compared to white rice because it contains large quantity of bio-active compounds like phenolic compounds, flavonoids, gamma oryzanol, ferulic acid, alpha tocopherol, gamma amino butyric acid (GABA), lignin, etc.[5]

Germinated Brown Rice (GBR)

The sprouted brown rice is obtained by soaking method. Brown rice soaking in water for 3 hours and leaving wet for 21 hours will produce budding. At the germination time, the nutrient quality, taste, enzyme activity and chemical compositions of rice changes. The quality, flavour and texture of unpolished brown rice increased through germination also essential nutrients like vitamins, minerals, fiber, ferulic acid, lysin, inositol, GABA, gamma oryzanol, tocotrienol increased[6] GBR alleviate health problems like DM, cardio vascular disease, hypertension, Alzheimer's, stress, depression, insomnia, dementia, colon cancer, constipation, etc.[7] Optimum amount of gamma amino butyric acid (GABA) obtained through sprouting. GABA is an inhibitory neurotransmitter, which reduce health risks and Presence of high GABA makes GBR popular. GBR contain two times higher content of GABA than normal brown rice and 10 times higher GABA than white rice.[7]

MATERIALS AND METHODS

Brown rice (1 kg) was collected from domestic market of Malappuram of Kerala state, India (2021 January).

Preparation of material: The rice grains were thoroughly washed and soaked in water overnight. Then leave it moist for 20-24 hours at 30^{o} C. The sprouting appeared within 2-3 days, shade dried it and grinded into powder.

Extraction of sample: 20 g powder was weighed and suspended in in 200 ml of water, ethanol solvents. Extraction was done by using Soxhlet apparatus for 5 hours.

GC-MS Analysis of Bioactive Compounds

Gas Chromatography-Mass Spectrometry (GCMS), is a combination of gas chromatography and mass spectrometry used to identify unknown samples and separate volatile and semi-volatile compounds. Ethanol extract of brown rice used for this technique. Gas chromatography combined with Shimadzu mass spectrometry (GC-MS- QP 2020 NX SHIMADZU). GC consist of a capillary column (DB- 5ms) of fused silica having film thickness of 0.25 micro meter. The carrier gas used was Helium at 1.0 ml/min flow rate. 1 micro litre sample was injected by autoinjector. The temperature of injector and detector was set at 250^{o} C. The primary temperature of column is 40^{o} C raised to 1000 C at 5^{o} C/min for 3 min, again raised temperature 250^{o}C at 10^{o} C/min. The ion source was set at 200^{o} C. Whole process of GC carried out at 39 mins. For the Mass Spectrometric (MS) detection were adjusted to an ionization energy of 70 eV. Temperature of electron impact ion source was set at 260^{o} C and transmission line at 280^{o} C. The mass scan range was m/z 40-300 for volatile compounds, since their molecular weight is low. The ethanolic extract of brown rice was qualitatively detected by a Shimadzu GC-MS solution (version.4) software.

Identification of Compounds

The mass spectrum of GC interpreted by using Databases of National Institute of Standard and Technology (NIST4) and WILEY9. It was by the comparison of spectrum of a known component with spectrum of unknown component stored in inbuilt library.

Antioxidant property of germinated brown rice (Oryza sativa L.)

Estimation of enzymatic antioxidants

a) Estimation of SOD

Estimation of SOD was based on inhibition of nitro-blue formation of superoxide ion by plant extracts, measured spectrophotometrically at 560 nm. 3 ml samples were taken, which contain 0.02 ml extract, 0.2 ml EDTA, 0.1 ml NBT, 0.05 of riboflavin and 2.64 ml of phosphate buffer. Control tubes were set up, where DSMO was added instead of plant extract. All tubes were vortexed, initial optical density measured at 560 nm. Tubes were illuminated using fluorescent lamp for 30 minutes. Then absorbance measured at 560 nm. Illumination was indicative of superoxide ion scavenging activity.

b) Estimation of hydrogen peroxide scavenging assay

The ability of plant extract to scavenge hydrogen peroxide was estimated by method of *Ruch et.al* (1989). Solution of H_2O_2 (40mM) in phosphate buffer. Plant extracts at concentration of 10mg/micro litre was added to H_2O_2 solution (0.6ml), total volume was made up to 3ml. Absorbance of solution was recorded at 230 nm. Blank solution containing phosphate buffer without H_20_2 was prepared.

Estimation of non-enzymatic antioxidants

a) Estimation of total flavonoid content

ALUMINIUM CHLORIDE METHOD

Aluminium chloride reacts with C-4 keto group and either the C-3 or C-5 hydroxyl groups of flavones and flavanols and forms acid-labile compounds with flavonoids. 0.5 ml sample added into test tube containing 1.25 ml distilled water. Then 0.075 ml of sodium nitrite was added and allowed to stand for 5 minutes. 0.15 The mixture was diluted by 0.75 ml distilled water. The absorbance of solution at 510 nm was measured. The resultant flavonoid content was expressed in milligram catechin.

b) Estimation of total phenolic compounds

FOLIN-CIOCALTEU METHOD

Phenol reacts with phosphomolybdic acid Folin-ciocalteu reagent in alkaline medium, produce bluish coloured complex. Pipetted out different volumes of (0.2-1ml) of standard into test tube and made up to 3 ml with distilled water, added 0.5 ml folin-ciocalteu reagent. After 3 minutes, added 2 ml of 20 % sodium carbonate to each tube, mixed well, boiled for exact 1 minute, cooled and read absorbance at 660 nm colorimetrically. Prepared calibration curve to different concentrations.

Estimation of antioxidants by DPPH assay

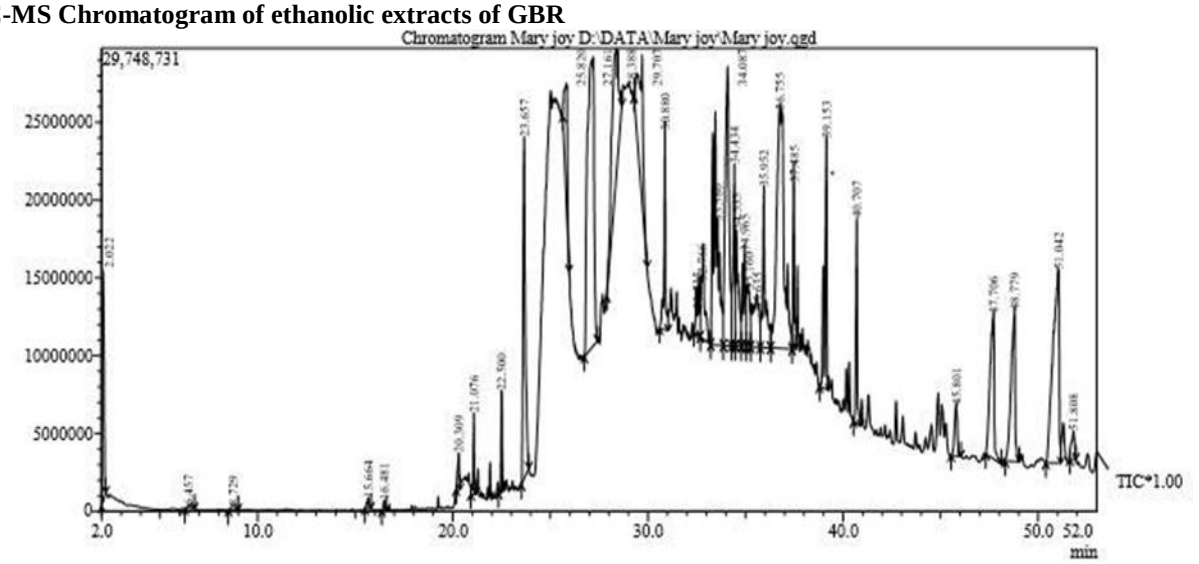
Antioxidants inhibit lipid oxidation, so scavenging of DPPH radical hence determine free radical scavenging ability.it is widely used method. 0.1 mM solutions of DPPH in methanol prepared, 1ml of this solution added into 3 ml extract in methanol (concentrations 50,100,200,400 &800 micro gram/ml). shaken vigorously allowed to stand for 30 minutes at room temperature. Read absorbance at 517 nm. If the absorbance value is lower, the more is the free radical scavenging capacity. The capacity of radical scavenging calculated by following formula

DPPH scavenging effect (% of inhibition) ={(A0-A1) /A0) *100}

Where, A0 is absorbance of control reaction, A1 is absorbance in presence of all extract sample and reference. All tests performed in three times and results were averaged.

RESULTS AND DISCUSSIONS

GC-MS Chromatogram of ethanolic extracts of GBR

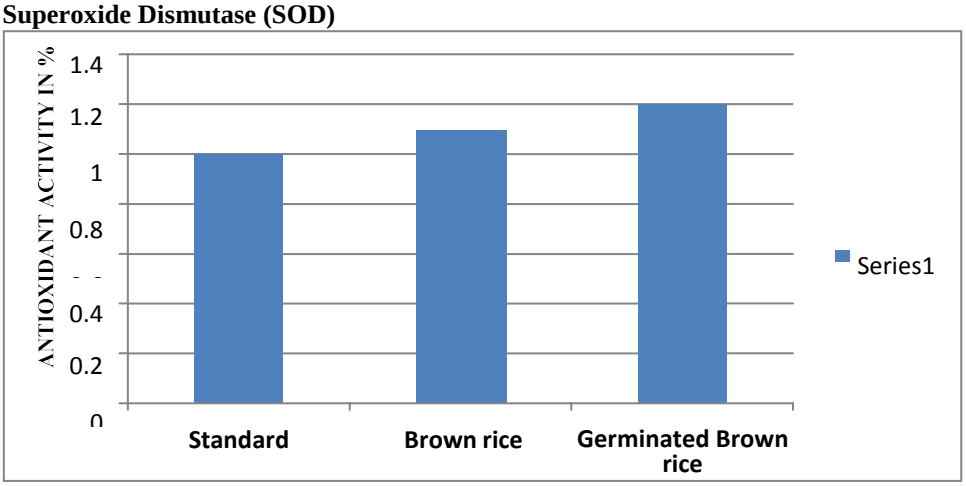


Mass spectrometry of ethanolic extract of GBR

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

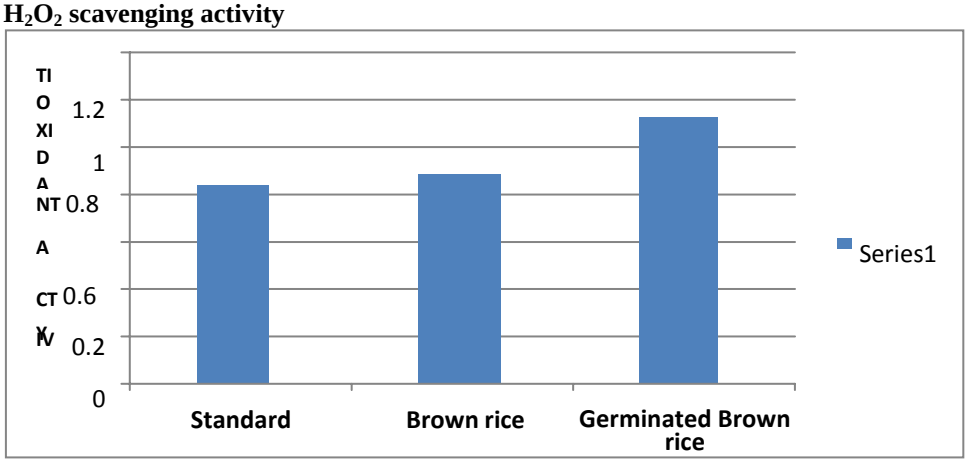
Antioxidant activity analysis

Estimation of Superoxide Dismutase (SOD)



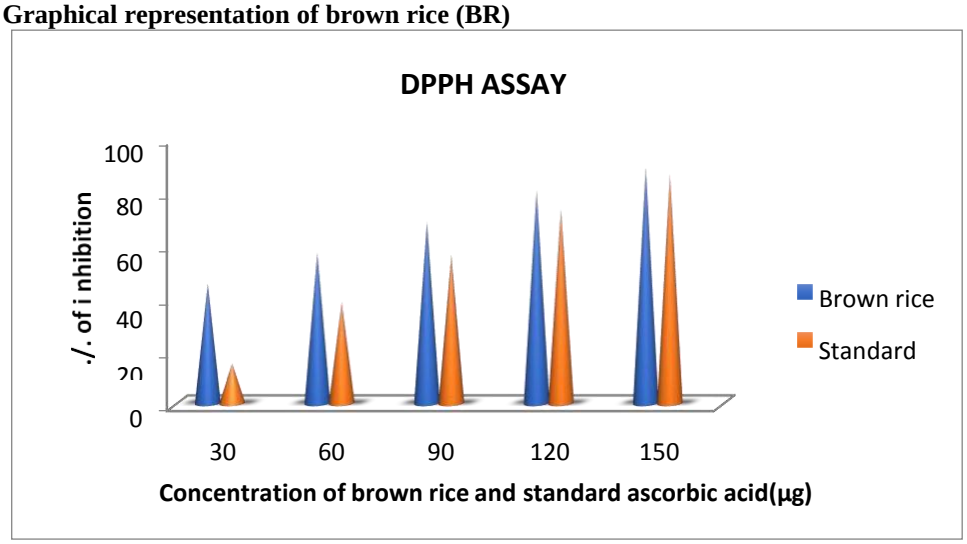
The result shows the GBR contain higher activity of SOD, compared with normal brown rice.

Estimation of H_2O_2 scavenging activity

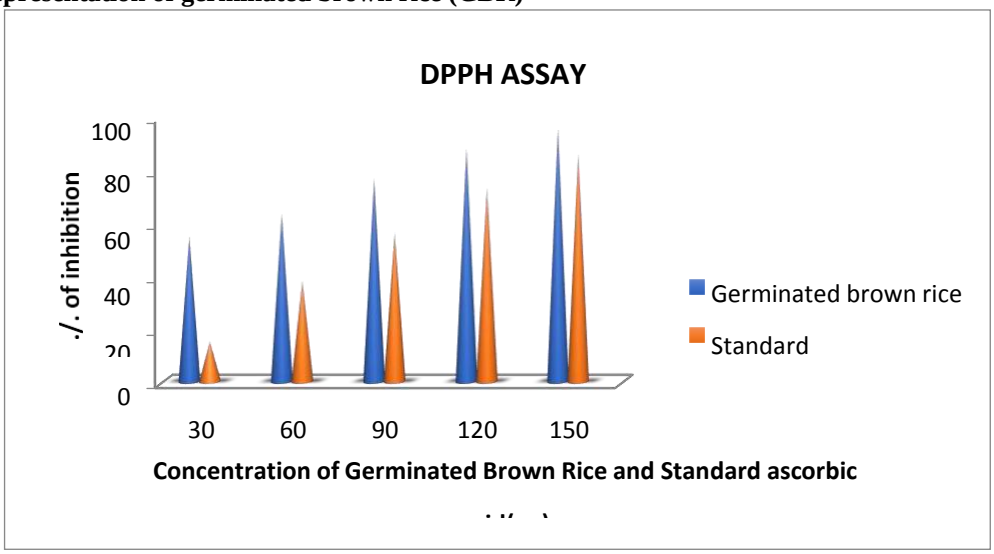


From the graph, the H_2O_2 scavenging capacity of GBR was higher when compared with non- germinated brown rice.

DPPH assay: Graphical representation of brown rice (BR)

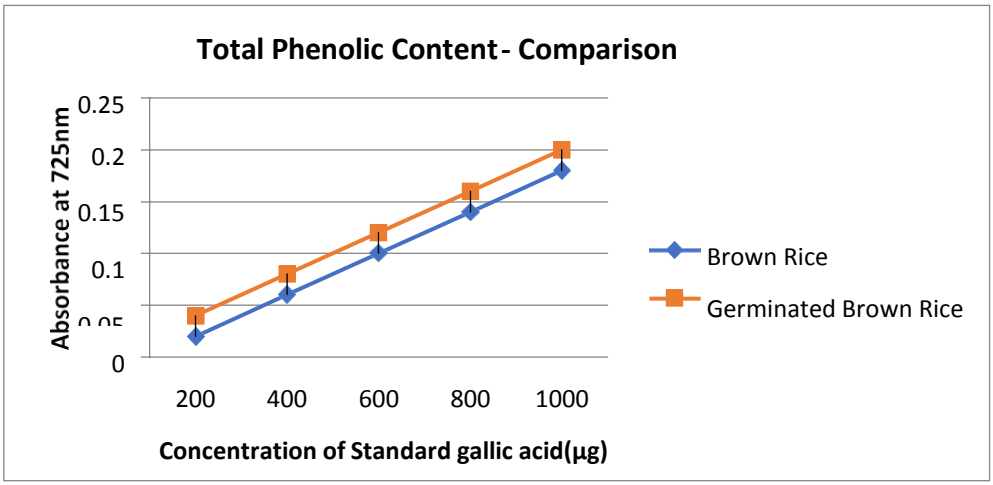


Graphical representation of germinated brown rice (GBR)



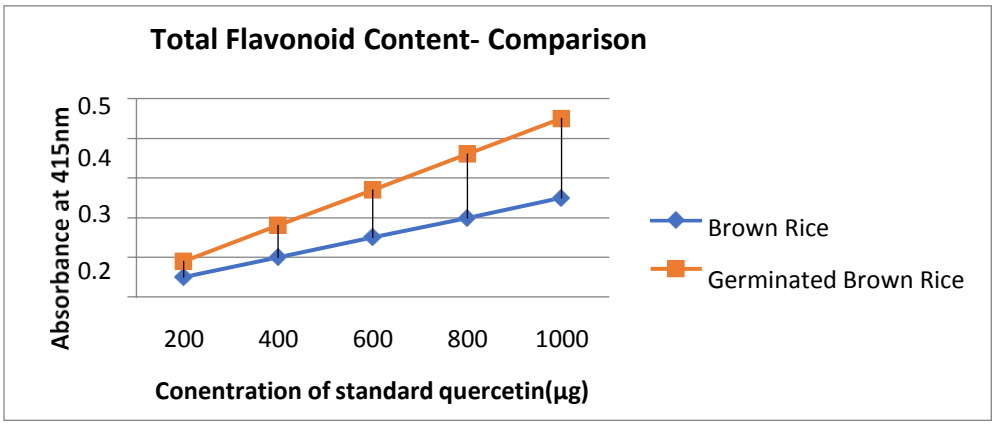
The results shows that the percentage of inhibition rate is more in GBR when compared to non germinated brown rice.

Total phenolic content



From this result shows that the total phenolic content is more in germinated brown rice than non-germinated brown rice.

Total flavonoid content



Results shows that the total flavonoid content is higher in germinated brown rice than non- germinated brown rice.

CONCLUSION

In this study, the ***Antioxidant property of germinated brown rice*** was determined and also ***Comparison of GBR with non-germinated brown rice*** were conducted**.** The germinated brown rice contains high levels of bioactive compounds like phenolics, flavonoid, flavanols, anthocyanidins, vitamins and more amino acid content than non-germinated brown rice, this makes the GBR more potent in antioxidant activity.

The bioactive compounds of GBR were evaluated by GC-MS analysis thus obtained chromatogram of ethanolic extract of GBR. The antioxidant property of GBR was assessed by using estimation of enzymatic (SOD, H_2O_2 scavenging activity) and non-enzymatic (total phenolic content, total flavonoid content, DPPH scavenging assay) activities. The free radical scavenging capacity of GBR was determined by DPPH scavenging and H_2O_2 radical scavenging assays.

In both assays GBR shows very high scavenging activity than standard and non-germinated brown rice. Thus, it can be concluded that germinated brown rice (Oryza sativa L.) have potent antioxidant property and this is higher than non-germinated brown rice.

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