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COMPARITIVE STUDY ON ANTIOXIDANT ACTIVITY, TOTAL PHENOL, FLAVONOID CONTENT IN GBR AND BR, GABA DETERMINATION AND ITS ACTIVATION IN INHIBITION OF α-AMYLASE ACTIVITY

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Abstract

The brown rice is a whole grain. It contains all part of grains provide fiber and several vitamins and minerals. Brown rice has many health benefits and reduce the risk of diseases. Germinated brown rice contains more bioactive components, such as ferulic acid and GAMA (gamma aminobutyric acid). It is powerful neurotransmitter, boosting the central nervous system's ability to support good sleep and reduce stress. Antioxidants prevent the damage caused by oxidation by scavenging free radicals from the body cells provides immunity to the body. Antioxidant activities of phenolic acids in the brown rice protect the body cells against oxidative damage, reduce the risk of disease. The acidic and basic catalysed hydrolysis in germinated brown rice (GBR) by the total phenol and flavonoid content responsible for antioxidant activity. GBR contains an stronger free-radical scavenging effect and a greater concentrations of bioactive compounds including total phenols and total flavonoid. GABA is a natural brain relaxant that makes relax during stress after the neurotransmitter is released. GABA activity leads to control anxiety, depression and mood disorders. Alpha-amylase ptyalin is produced in the digestive system of humans and other mammals by salivary glands acts upon the starches. The starches can be broken down to maltose by ptyalin during digestion involves in the function of catalysing the hydrolysis of start into maltose and dextrin.

Key words: Brown rice, GBR, Antioxidant activity, GABA.

INTRODUCTION

Brown rice is a rich source of phenols and flavonoids, two types of antioxidants that help reduce damage to cells and reduce the risk of premature aging. Brown rice also contains high levels of magnesium, which can help make you less vulnerable to heart disease, stroke, better functioning of the cardiovascular system, digestive system, brain, and nervous system. It is loaded with powerful antioxidants which provide relief from a range of conditions such as hypertension, unhealthy levels of LDL cholesterol, stress, mental depression, and skin disorders. Germinated rice is easier to digest and has more bio-available nutrients. Health benefits of consuming germinated brown rice includes improved brain function, relief of some menopause symptoms, lower blood pressure, prevent headaches, relieve constipation, regulate blood sugar. 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. Antioxidant activity denotes the ability of a bioactive compound to maintain cell structure and function by effectively clearing free radicals, inhibiting lipid peroxidation reactions, and preventing other oxidative damage. Antioxidant Composition of Rice. the antioxidant compounds in rice were classified into six groups: phenolic acids, flavonoids, anthocyanins and proanthocyanidins, tocopherols and tocotrienols (vitamin E), γ-oryzanol, and phytic acid. Results showed that the antioxidant activity of the Se-GBR protein is higher than that of GBR under all treatment conditions. Heating improved the antioxidant activity of proteins. germinated brown rice (Oryza sativa L.) (GBR) for total phenols, flavonoids and antioxidant and allelopathic activities. The relationship between GABA and α-amylase activity was investigated by treating barley aleurone with exogenous GABA.

GBR

Germinated brown rice has been suggested as an alternative approach to mitigate highly prevalent diseases providing nutrients and biologically active compounds. During germination, significant changes occur in biochemical, nutritional, and sensory characteristics. Germination process generally results in improved levels of vitamins, minerals, fibers, and phytochemicals such as ferulic acid, gamma-aminobutyric acid, and γ-oryzanol. Consumption of germinated brown rice is receiving increasing attention supported by scientific evidence on its beneficial health effects reducing the risk of diseases such as obesity, cardiovascular diseases, type-2 diabetes, and neurodegenerative diseases. Germinated brown rice has been identified as a natural and inexpensive substitute of conventional white rice to improve nutritive and health status of a large population.

Anti-oxidant activity

Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Antioxidants are found in many foods, including fruits and vegetables. plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxides. Traditional herbal medicines, dietary foods were the main source of antioxidant for ancient peoples that protected them from the damage caused by free radicals.

Anti-depression activity

Antidepressants are medications that can help relieve symptoms of depression, social anxiety disorder, anxiety disorders, seasonal affective disorder, and dysthymia, or mild chronic depression, as well as other conditions. They aim to correct chemical imbalances of neurotransmitters in the brain that are believed to be responsible for changes in mood and behavior. It inhibits the action of monoamine oxidase, a brain enzyme. Monoamine oxidase helps break down neurotransmitters, such as serotonin. If less serotonin is broken down, there will be more circulating serotonin. In theory, this leads to more stabilized moods and less anxiety.

Phytochemical screening

In this method, aqueous and organic extracts are prepared from those plant samples that are the reservoir of secondary metabolites, such as leaves, stems, roots, or bark. The plant extracts are then analyzed for the presence of secondary metabolites like alkaloids, terpenes, and flavonoids. Phytochemical screening was carried out on the leaf extracts using different solvents such as aqueous solvents, acetone, ethanol, petroleum ether and chloroform. secondary metabolites such as tannins, saponins, phenols, terpenoids, alkaloids, glycosides, and cardiac glycosides etc. Based on the results, each secondary metabolite has its own biological function. Studies reported that terpenes exert effectiveness in human disease and prevention. The presence of alkaloids, are responsible for analgesic, antispasmodic and bactericidal activity, whereas for saponins, shown to have cholesterol binding properties and hemolytic activity. Plant steroids play a vital role in cardiotonic activities.

MATERIALS AND METHODS

1.SAMPLE COLLECTION

The samples was collected from fields at Thrissur, Kerala, India during January, 2022.

2. SAMPLE PROCESSING

Samples was cleaned thoroughly and dried, then ground to powder using blender for making thin powder. Germinated brown rice was dried and make into thin powder by blending and separated by sewing.

3. PREPARATION OF SOLVENT EXTRACTS

PREPARATION OF AQUEOUS EXTRACT

Aqueous extract was prepared by taking 5gm sample powder dissolved with 50ml of distilled water by occasionally shaking for 24 hours. The extract was filtered through Whatmann No.1 filter paper and then concentrated to the one fourth of the original volume by evaporation at room temperature.

PREPARATION OF ETHANOL EXTRACT

About 5gm of sample powder was dissolved in 50ml of ethanol and kept it in a shaker for 24 hours. The extract was filtered through Whatmaan No.1 filter paper.

4. QUALITATIVE PHYTOCHEMICAL ANALYSIS

Preliminary screenings of the different extracts were performed to investigate the presence or absence of the different phytochemical constituents.

DETECTION OF CARBOHYDRATES

Extracts were dissolved individually in 5ml of distilled water and filtered. The filtrates were used to test for the presence of carbohydrate.

Molisch's Test;

Filtrates were treated with 2 drops of alcoholic α- naphthol solution in attest tube and add concentrated sulphuric acid along the sides of the tube without mixing. Formation of the violet ring at the junction indicates the presence of carbohydrates.

Benedict's test;

Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugar.

Fehling's Test;

Filtrates were hydrolysed with dil. HCL, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugar.

DETECTION OF ALKALOIDS

Extract were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's test;

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide).

Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wager's Test ;

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide).

Formation of brown/reddish precipitate indicates the presence of alkaloids.

DETECTION OF FLAVANOIDS

Alkaline Reagent Test:

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test:

Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

DETECTION OF GLYCOSIDES

Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test:

Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution.Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides.

Legal's Test:

Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red color indicates the presence of cardiac glycosides

DETECTION OF PHENOLS

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

DETECTION OF SAPONINS

a) Froth Test:

Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b) Foam Test:

0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

DETECTION OF TANNINS

Gelatine Test:

To the extract, 1% gelatine solution containing sodium chloride was added.

Formation of white precipitate indicates the presence of tannins.

DETECTION OF PROTEIN:

Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

DETECTION OF AMINO ACIDS

Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid..

DETECTION OF PHYTOSTEROLS

Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes. Liberman Burchard's test; Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

HPLC

HPLC analysis of GABA

HPLC is the most popular method for analyzing amino acid components, which have currently gained attention due to the boom in health foods. This page discusses mainly the detection methods used to analyze amino acids.

DETECTION METHODS:

PRE COLUMN DERIVATION:

Using UV detection for amino acids in most cases requires using the absorption of the carboxyl group (-COOH) in the 200 to 210 nm range. Some amino acids with benzene rings can also be detected in the 250 to 280 nm range, but in general, they are difficult to analyze as-is with sufficient sensitivity and selectivity. Consequently, derivatization methods have long since been usedSince many amino acids contain amino groups (-NH2 and -NHR) in their structures, a derivatizing reagent that selectively reacts with the amino group is used.

ADVANTAGES:

In general, reagent consumption rates can be minimized by specifying a small reaction system.Allows increasing sensitivity by using more expensive reagents that provide lower background levels (than post-column derivatization).Even if unreacted derivatizing reagent is detected, as long as it is separated in the column, it does not cause a problem.

POST COLUMN DERIVATION:

The post-column derivatization method involves separating the amino acids in the column, then delivering and mixing the derivatizing agents with amine groups in the given sampes.

ADVANTAGES:

Can be automated offering excellent quantitative performance and reproducibility. Since sample components are separated before the reaction, reaction efficiency is less prone to sample matrix effects, enabling it to be used for a wide range of samples.

INHIBITION OF α-AMYLASE ACTIVITY

The α-amylase inhibition assay was performed using 3,5-dinitrosalicyclic acid(DNSA). The GBR extract was dissolved in minimum amount of 10% DNSA and was further dissolved in buffer Na2HPO4 at PH 6.9 to give concentration ranging from 10 to 1000µg/ml. A volume of 200µl of the extract and was incubated for 10 min at 30C. Thereafter 200µl of the starch solution was added to each tube and incubated for 3min. Add 200µl of DNSA reagent and was boiled for 10min water bath at 85 to 90C.The mixture was cooled and diluted with 5ml of distilled water and the absorbance was measured at 540nm .The blank with 100% enzyme activity was prepared by replacing the extract with 200µl of buffer. A blank reaction was similarly prepared using extract at each concentration in the absence of enzyme solution. A positive control sample was prepared by using Acarbose (100µg/ ml-2µg/ml) and the reaction was performed similarly. The alpha amylase inhibitory activity was expressed as percent inhibition was calculated.

Result and discussion

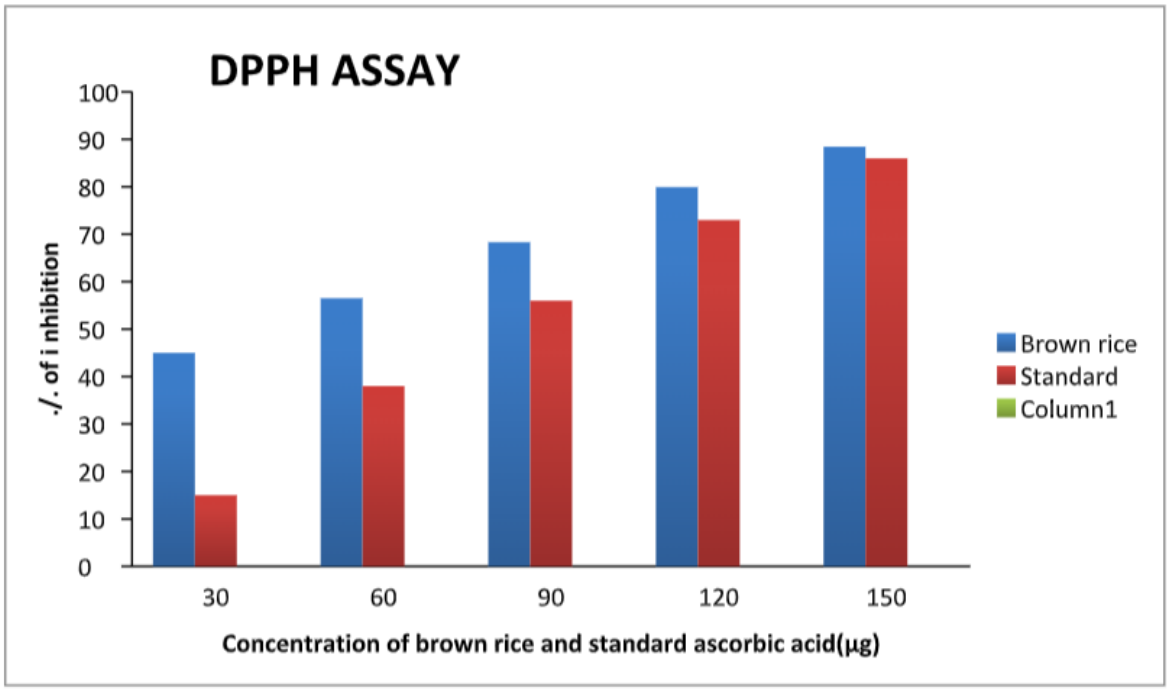
SL. NO	Compounds	GBR Aqueous	GBR Ethanol	BR Aqueous	BR Ethanol
1.	CARBOHYDRATES	+	+	+	+
2.	ALKALOIDS	+	+	+	+
3.	FLAVONOIDS	+	+	+	+
4.	GLYCOSIDES	+	+	+	+
5.	PHENOLS	+	+	+	+
6.	SAPONINS	+	+	+	+
7.	TANNINS	+	+	+	+
8.	PROTEINS	+	+	+	+
9.	AMINOACIDS	+	+	+	+
10.	PHYTOSTEROLS	+	+	+	+

The phytochemical analysis of GBR and brown rice shows that the

presence of all phytochemicals .

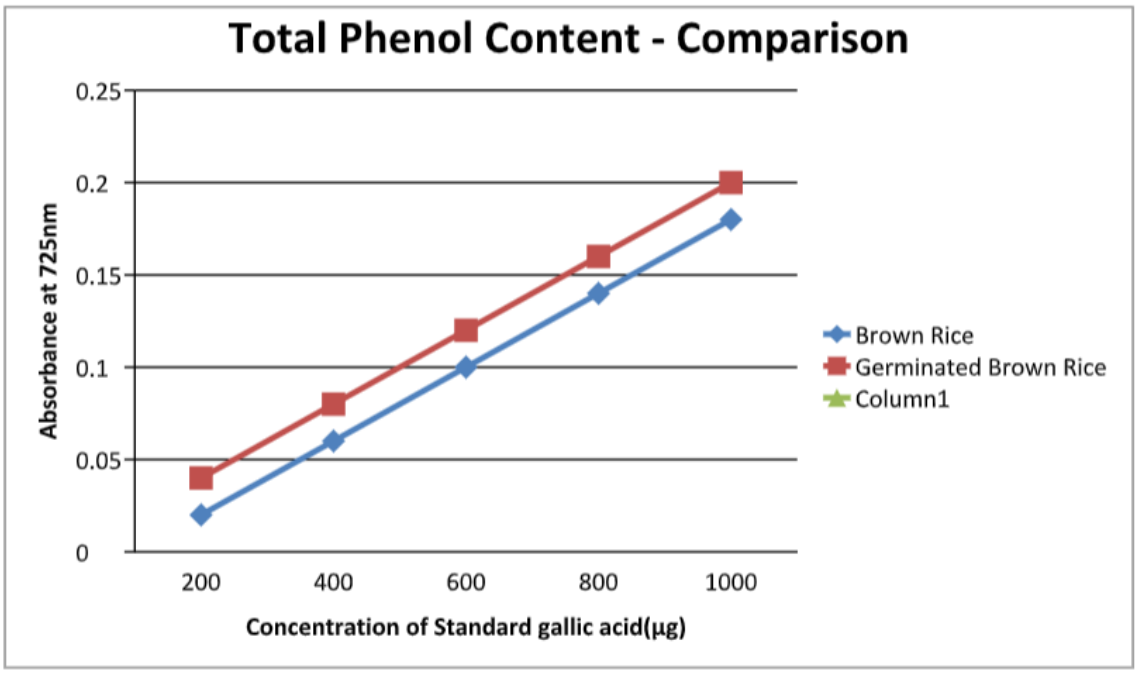
ANTIOXIDANT ACTIVITY

GRAPHICAL REPRESENTATION – BROWN RICE:



GRAPHICAL REPRESENTATION:

TOTAL PHENOL CONTENT

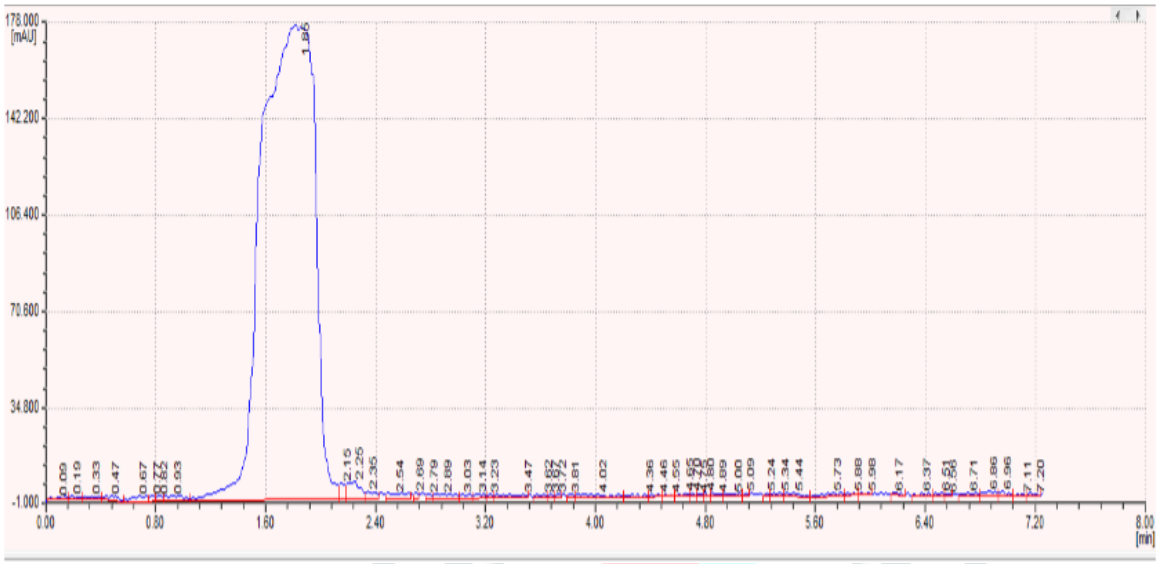


From this result shows that the total phenolic content is more in germinated brown rice than non germinated brown rice.

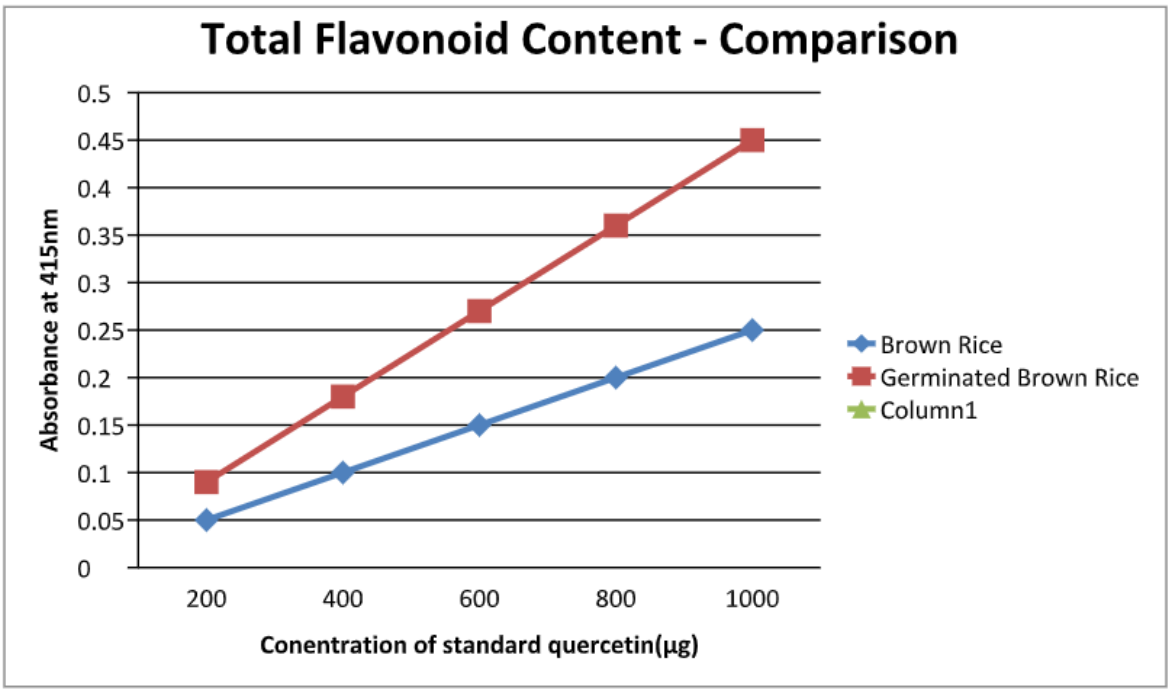
TOTAL FLAVONOID CONTENT:

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

HPLC



HPLC chromatogram of gamma aminobutyric acid with standard GABA and



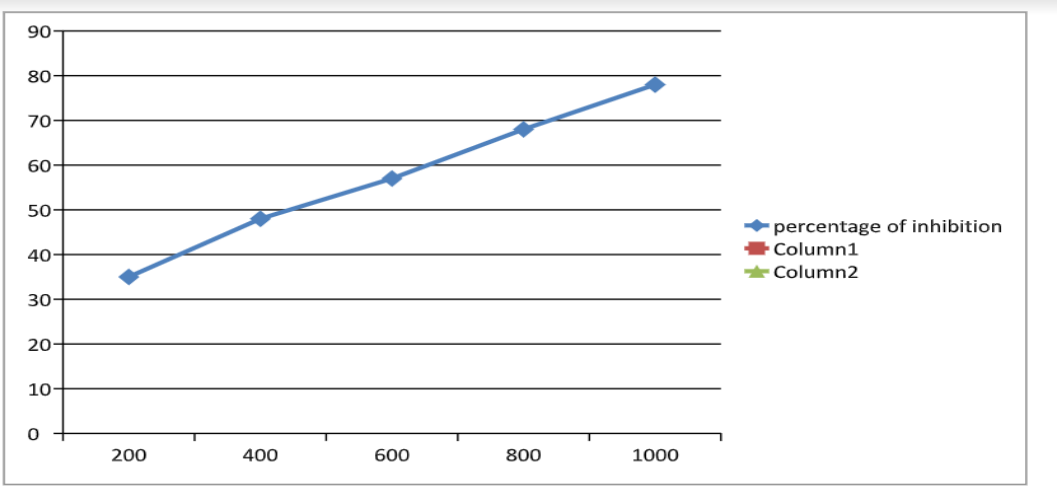
GABA in ethanolic extract of germinated brown rice.

INHIBITION OF α-AMYLASE ACTIVITY:

GRAPHICAL REPRESENTATION

ALPHA AMYLASE INHIBITION ACTIVITY OF GERMINATED BROWN RICE:

From the graphical representation the inhibition of alpha amylase by germinated brown rice . At 1000 µg , germinated brown rice shows the highest level of inhibition of alpha amylase with 79./.



Summary and conclusion:

The results of Secondary metabolites and Antioxidant activities germinated brown rice has more antioxidant activity than non-germinated, among the secondary metabolites assessed, total flavonoids were higher at all concentrations studied compared to phenolic. Higher values obtained with flavonoid signify that flavonoids are the major class of phenolic compounds in brown rice. After soaking and incubation, GABA content in germinated brown rice was generally higher than in non-germinated brownrice. This indicated that the storage protein in grains was decomposed at least partially and supplied to the growing part of the seedlings and within this process glutamate decarboxylase enzyme was activated which converted glutamic acid to GABA.

GABA in germinated brown rice have the activity of inhibition of alpha amylase .It is very effective for diabetics.So it has been concluded that germinated brown rice has higher GABA contentand also contain many bioactive components. It is very effective for inhibiting the alpha amylase activity in Type 1 diabetic mellitus.

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