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EVALUATION OF ANTIOXIDANT ACTIVITY OF GAMMA ORYZANOL

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ABSTRACT

Rice bran oil contains a wealth of nutritionally significant phytoceuticals like oryzanol, tocotrienols, tocopherols, and other compounds, rice bran oil stands out among other edible oils. One of the ingredients in rice bran oil, oryzanol, has prospective applications in the pharmaceutical, cosmeceutical, and nutraceutical industries. It functions as a natural antioxidant in rice bran oil, where it is found at a concentration of 1% to 2%. In the current investigation, γ-oryzanol was extracted from rice bran oil in a manner that ultimately produced a high yield product. In order to create soap stock, rice bran oil was first treated with alkali and then treated with acid. Following this, the extracted product was washed with distilled water before being crystallized even more. The product was passed through crystallization solvents and then incubated at 4°C in a deep freezer to achieve crystallization. These procedures produced pure γ-oryzanol. The quantization of γ-oryzanol in its crystalline state revealed a concentration of 36.2% at 315 nm. γ-oryzanol is a component of several types of ferulic esters and phytosterols. The only thought to justify the use of γ-oryzanol in a variety of applications, such decreasing cholesterol levels in the human body, is its antioxidant capacity. Using three distinct methods—the DPPH scavenging assay, the nitric oxide scavenging assay, and the reducing power assay—this antioxidant activity was demonstrated in the current work. The findings demonstrated that γ-oryzanol has extremely high antioxidant activity, suggesting that it might be included in a person's diet to help prevent a variety of disorders like hypercholesterolemia and arteriosclerosis that are brought on by excessive cholesterol.

KEY WORDS:- scavenging, Superoxide radicals, Lipoxygenases, Peroxynitrite,Antioxidant.

INTRODUCTION

Reactive oxygen species (ROS) are produced as byproducts of oxygen metabolism and can play a variety of physiological roles, including cell signaling. However, ROS production is greatly increased by environmental stressors, such as UV radiation, ionizing radiation,

pollutants, and heavy metals, as well as xenobiotics, such as antiblastic drugs, which create an imbalance that damages cells and tissues (oxidative stress). A biological system's capacity to detoxify reactive products like oxygen reactive species (ROS) is out of balance, which leads to the condition known as oxidative stress. ROS are produced and accumulate in cells and tissues. Reactive oxygen species (ROS) are typically described as superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and singlet oxygen ($^1O_2$), which are produced by biological systems as metabolic byproducts. (sato et al., 2023) (Navaroo-Yeoes et al. 2014).Under both normal and pathological circumstances, mitochondria are the primary source of reactive oxygen species (ROS). $O_2^{\bullet-}$ can be created by cellular respiration, lipoxygenases (LOX) and cyclooxygenases (COX) during the metabolism of arachidonic acid, endothelial cells, and inflammatory cells (Al-Goboury et al., 2012).

There are two main sources of free radical generation: endogenous and external. Endogenous free radical production is caused by a number of factors, including age, excessive exercise, mental stress, inflammation, ischemia, infection, malignancy, and immune cell activation. Exposure to various substances such as chemicals, solvents, heavy metals (Cd, Hg, Pb, Fe, and As), medications (cyclosporine, tacrolimus, gentamycin, and bleomycin), smoked meat, used oil, and fat during cooking, alcohol, and radiations can all result in the production of exogenous free radicals (Valko et al., 2007) (Feri, 1997). Upon entering the body, these foreign substances undergo breakdown or metabolism, leading to the production of free radicals as a byproduct.

Oxidative stress is a dangerous process that can adversely impact a number of cellular components, including membranes, lipids, proteins, lipoproteins, and deoxyribonucleic acid (DNA), if free radicals and oxidants are present in excess (Dorge, 2002) (Young and Woodside, 2001). When there is an imbalance between the production of free radicals and the ability of cells to eliminate them, oxidative stress results. For example, too much hydroxyl radical and peroxynitrite can lead to lipid peroxidation, which harms lipoproteins and cell membranes. This will therefore result in the creation of conjugated diene compounds and malondialdehyde (MDA), both of which are known to be mutagenic and cytotoxic. Lipid peroxidation is a radical chain process that spreads swiftly, impacting a significant number of lipidic molecules (Frei, 1997). Additionally, oxidative stress can cause structural changes in proteins that may result in a decrease in or impairment of their enzymatic activity (Halliwell, 2007).

Natural antioxidants can delay the onset of many chronic diseases, shield the body from free radical damage, and stop food's lipids from oxidatively becoming rancid (Pryor, 1991). The potential of plant products as antioxidants against a range of diseases caused by free radicals has been the subject of recent investigations (Hou et al., 2003). Natural antioxidants, such as polyphenols found in food and medicinal plants, are gaining popularity as they may be able to counteract oxidative damage Silva et al., 2005. It has been established that phenolic components like flavonoids and phenolic acids are primarily responsible for the antioxidant activity of plant products (Pietta, 2000).

Extracted from rice bran oil, gamma-oryzanol is a phytosteryl ferulate combination with a broad range of biological activity. It has been claimed that gamma-oryzanol offers several health benefits, including improving the plasma lipid pattern, lowering total plasma cholesterol and raising HDL cholesterol levels, and preventing platelet aggregation. From our perspective, what's more intriguing is that gamma-oryzanol was found to demonstrate antioxidant qualities in in vitro systems, including pyrogallol autoxidation, lipid peroxidation induced by ferric ions in porcine retinal homogenate, and cholesterol oxidation accelerated by 2,2′-azobis(2-methylpropionamidine). Gamma-oryzanol has been suggested as a natural antioxidant to increase food stability due to its short-term safety.

MATERIAL AND METHOD

The first step in the extraction of oryzanol was rice bran oil, which was bought from the Indore, India. One tetra pack, containing 1L (910g) of rice bran oil, was purchased from Satguru Agro Rice Bran Oil. High purity chemicals were purchased from HiMedia Laboratory Pvt. Ltd.

Antioxidant activity of oryzanol

By using in-vitro techniques such as the DPPH scavenging activity, Nitric oxide scavenging activity, and reducing power test, the antioxidant activity of oryzanol was determined. Ascorbic acid was used as the standard and the test was carried out in triplicate. With minimal alterations, the DPPH test was carried out in accordance with Muthal et al., 2015's instructions. In short, 1 ml of a DPPH 0.1 mM solution was added to a 2 mg/ml oryzanol sample that had been produced in ethanol and emptied into several test tubes with variable concentration ranges from 10 to 100µg/ml. After shaking the mixture, it was incubated for 30 minutes at 370 C. OD was measured at 517 nm after 30 minutes (Muthal et al., 2015). A positive control was ascorbic acid. The scavenging activity of DPPH was determined by:

$$\frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where **$A_{control}$** represents reading of control reaction and **A_{test}** absorbance of the Oryzanol

With little changes, the nitric oxide scavenging activity was measured according to Alam et al., 2013's methodology. A solution of 2 mg/ml oryzanol was made in methanol, and 0.5 ml of various concentrations—from 10 to 500 mg/ml—was transferred into several test tubes. Two milliliters of a sodium nitropusside solution (made by dissolving 10 milligrams of sodium nitropusside in 0.5 milliliters of phosphate buffer saline, pH 7.4) was added to each test tube, and the mixture was then incubated for 150 minutes at room temperature. Following the incubation period, 0.5 ml of Griess reagent was added, and the mixture was incubated for 30 minutes. The mixture contained 1 ml of napthylenediamine dichloride (0.1% W/V) and 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) at room temperature for 5 minutes. At 546 nm, absorbance was measured. The nitric oxide radical inhibition calculated byequation:

$$\text{\% Inhibition of nitric oxide radical} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 represents absorbance before and A_1 represents absorbance after reaction has been take plane with Griess reagent

A 2 mg/ml oryzanol sample was prepared in methanol and added to each test tube at a concentration ranging from 10 to 500 mg/ml to conduct the reducing power assay. After adding 2.5 milliliters of potassium ferricyanide (1%) and normal saline (0.9%), the mixture was incubated for 20 minutes at 50 degrees Celsius. Following incubation, 2.5 ml of cold TCA (10%) was added, and the centrifuge was run at a high speed for 10 minutes. To 2.5 ml of supernatant, 2.5 ml of distilled water was added. After five to ten minutes, 0.5 ml of 1% ferric chloride was added, and the absorbance at 700 nm was measured. The plot of absorbance versus sample concentration was used to calculate the antioxidant activity.

RESULTS AND DISCUSSION

The separated γ-Oryzanol was collected prior to crystallization, and it had a thick, stick-like, yellowish-brown appearance.

Drying of γ – oryzanol:-The product was heated to temperatures ranging from 80ºC to 140ºC in order to facilitate drying. When the temperature hit 138ºC, it first had a black, oily appearance before stopping to boil and turning dark brown.

Crystallization of γ-oryzanol: The product underwent crystallization for purifying purposes following its drying process. After cooling at 0°C to 4°C, the product began to crystallize in 5–10 minutes, and the crystals grew quite quickly. When crystallization first began, the crystals had a cream-colored hue. However, consistent crystallization solvent washing caused the crystals' color to change white.Oryzanol quantization: The amount of oryzanol in the product was ascertained by employing spectrophotometric analysis at a wavelength of 314 nm. At 315 nm, the absorbance of the crystalline γ-oryzanol was measured and found to be 0.612. Based on the absorbance, the crystalline sample's γ-oryzanol content was determined to be 35.61% using a formula that included the γ-extinction coefficient of oryzanol.

$$\text{Content of oryzanol (\%)} = \frac{\text{Absorbance of sample (E)} \times \text{Volume of Hexane used}}{\text{Weight of sample (W)} \times \text{Extinction coefficient of sample (356)}}$$

Where absorbance of gamma oryzanol sample: 0.612, Volume of Hexane 10 ml, Weight of sample 50 mg

Antioxidant activity of oryzanol:- Figure 1 depicts our findings, which demonstrate the correlation between oryzanol concentration and DPPH activity. It was discovered that the DPPH scavenging activity was concentration dependant. The percentage of scavenging activity was 6.85% at a concentration of 10 µg/ml, and it climbed to 63.88% when the concentration was elevated to 100 µg/ml. In 2005, Akiyama and colleagues investigated the ability of γ-oryzanol constituents—cycloartenyl ferulate, 24-methylenecycroartanyl ferulate, β-sitosteryl ferulate, and campesteryl ferulate—to scavenge free radicals. These constituents were found by NMR and MS analysis. The scavenging activity was shown to be equivalent to that of ferulic acid, an active component of γ-oryzanol that is responsible for the DPPH scavenging action (Akiyama et al., 2005). 2015 saw the observation by Muthal et al. that 100 µg of γ-oryzanol and ascorbic acid showed 65.56% and 64.88% inhibition, respectively.

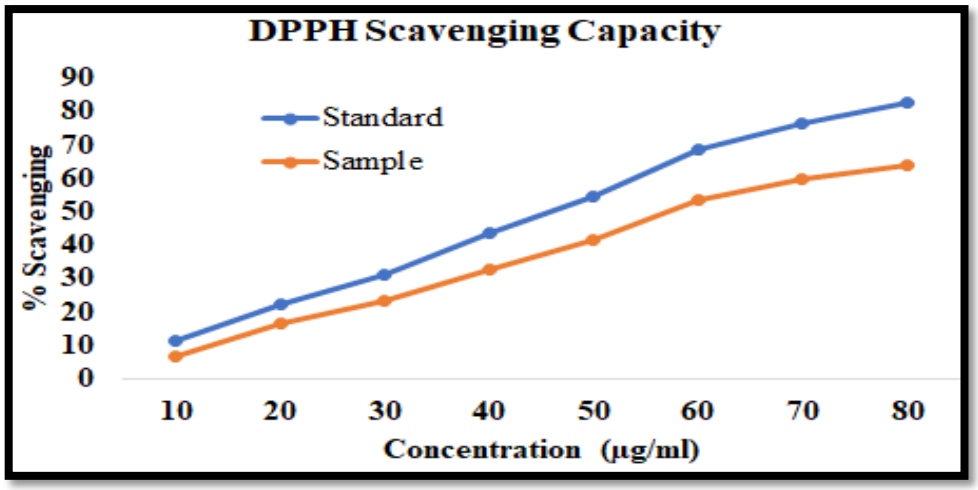

Figure 1:- DPPH Scavenging Capacity

The nitric oxide activity of γ-oryzanol is depicted in Figure 2. Plotting indicates that an increase in γ-oryzanol concentration during the experiment is accompanied by an increase in antioxidant activity, leading us to hypothesize that include γ-oryzanol in the human diet could effectively combat a variety of diseases. For the nitric oxide assay, the computed IC50 value is 62.83 (muthal et al., 2015). According to Juliano et al. (2005), gamma-oryzanol can operate as a stabilizer of lipidic materials since it increases the stability of oil that can undergo lipid peroxidation and prevents lipid peroxidation triggered by AMVN. According to Voraratn et al. (2010), the DPPH and FRAP assays were used to determine the high antioxidant activity of rice bran oil and rice bran oil emulsion. A considerable level of

vitamin E and gamma oryzanol were found in rice bran oil. The antioxidant activity of native rice bran oil and medium chain fatty acid (MCFA) rich rice bran oils was compared by Sengupta et al. (2015). The order of the different MCFA rice bran oils' antioxidant activity is as follows: rice bran oil rich in caprylic acid, rice bran oil rich in capric acid, rice bran oil rich in lauric acid, and rice bran oil (RBO).They added that the reduction in unsaturated fatty acid levels brought about by interesterification with MCFA is the cause of the increased antioxidant activity of MCFA rich RBO. When Tuncel et al. (2011) investigated the antioxidant activity of rice milling fraction, they found that unpolished rice had higher antioxidant activity than white polished rice and that whitening and polishing the rice reduced its gamma-oryzanol content by 94%.

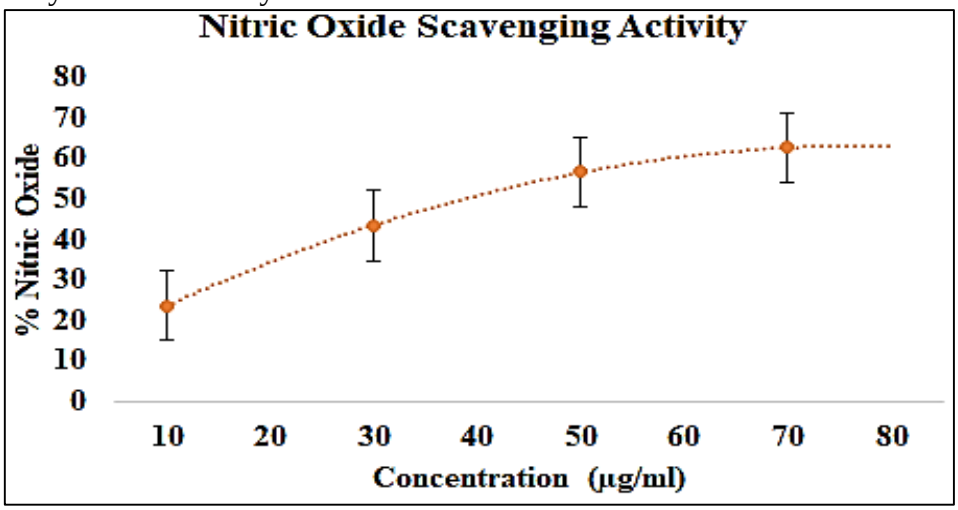

Figure 2:- Nitric Oxide Scavenging Activity

Figure 3 shows the γ-oryzanol's decreasing power activity. The outcomes of this activity are comparable to those of other antioxidant activities. It also indicates that elevating the γ-oryzanol concentration might benefit the body. The color of the sample tubes increased as the oryzanol concentration increased during the reducing power assay. It is thought that the antioxidant molecule forms a colored complex with the test's components during the reducing power assay. Therefore, the reaction mixture's color will increase with the amount of antioxidant ingredient present. The reaction mixture won't develop this color if there are no antioxidant compounds present. Oryzanol has very strong antioxidant action as evidenced by the increased color production in increasing order. According to research by Pengkumsri et al. (2015), hexane and the Supercritical Fluid Extraction method produced a higher yield of gamma-oryzanol than the Hot Pressed and Cold Pressed procedures. The rice bran oil extracted with hexane also had higher levels of antioxidants and phytochemicals.

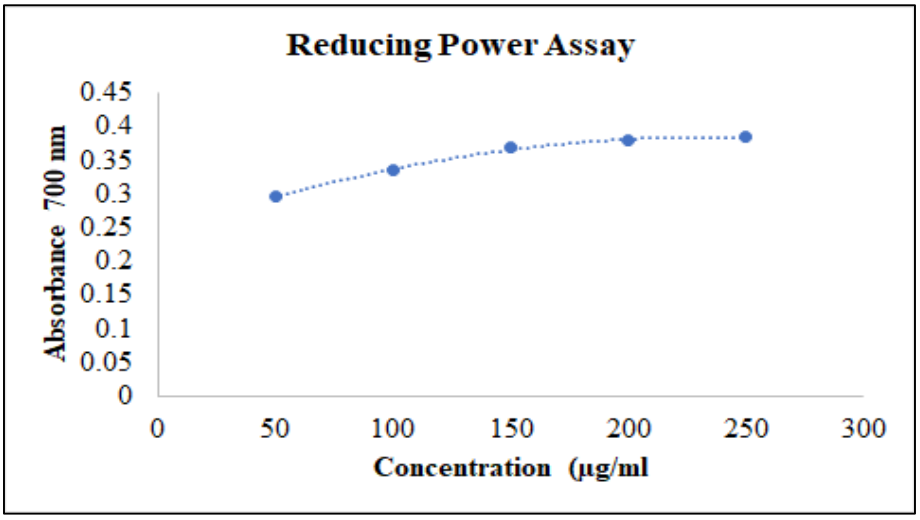

Figure 3:- Reducing Power Activity

CONCLUSION: -

Extracted from rice bran oil, gamma oryzanol was shown to have antioxidant activity that appeared to increase with concentration. Because it prevents cell damage and lessens the likelihood of free radical production. According to the study's conclusion, it should be regarded as a reliable source of oryzanol if it coincides well with the findings of other authors.

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