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Investigation of Antioxidant Activity of Gamma Oryzanol of Rice Bran

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ABSTRACT

The number of people over the age of 60 on the world stage is projected to increase twice as fast as in the past four years. In terms of longer life expectancy, human body cells are more prone to chronic diseases such as diabetes, heart disease and cancer due to lack of essential nutrients. As the condition is reaching an epidemic in many parts of the world, nutritional diet is demonstrated as the most important non-genetic contributor to human health. In fact, components present in many naturally occurring foods have been recognized to play important roles as promoters or inhibitors of these diseases. Thus, these photochemicalss present in their natural form may help as a major group component with beneficial effects in preventing or delaying the onset of nutritional diseases in specific population groups. Knowing how these components affect the health of human life will be possible only when we get a better understanding of the molecular basis of these components.

INTRODUCTION

Rice bran oil (also known as the extracted product of rice bran) is extracted from the treatment of germ and inner husk of rice, commonly called rice bran. The rice bran consisting about 15–25% of oil and (unlike oat bran) negligible amounts of water-soluble glucans and larger amounts of insoluble dietary fibre in it. For high smoke points of 235 °C (450 °F) and its mild flavour, making it suitable for high-temperature cooking methods such as stir frying and deep frying. It is popular cooking oil in several Asian countries, including Japan and China Many people have replaced their usual cooking oils with rice bran oil due to this higher nutritional value [1,2].

In recent years, *Oryza Sativa* (Rice) bran oil (RBO) is gaining surmount importance on account of its balanced fatty acid profile and rich source of commercially and nutritionally important antioxidative and disease-preventative phytochemicals such as, ferulic acid, its esterified derivative (oryzanol), and unsaponifiable components such as to copherol, tocotrienol, and vegetable sterols[Literature reports have suggested that RBO constituting of bioactive polyphenols exhibit anti-oxidative, hypo cholesterol emic, antiatherogenic, antidiabetic, cancer chemo-preventive and immune potentiation properties[3,4]. The isolation of bio-active principles from crude RBO (cRBO) has been carried out by preparative HPLC, calcium ion induced precipitation of anionic micellar aggregates and silica-based continuous chromatography combined with multistage of crystallization. However, some serious limitations of these techniques have been exposed, including low productivity, use of chlorinated or aromatic toxic solvents like benzene, multi-stage processes and non-reproducibility of the methods to production scale [5,6].

Antioxidant Activity of γ-Oryzanol

In order to establish the beneficial effects of Orz in the antioxidant defence of cellular systems, it is important to consider that dietary antioxidants are essential for maintaining normal cellular functions and to ensure body homeostasis. Nevertheless, the regulation of a redox mechanism through dietary means is currently gaining considerable traction in the field of human and food sciences. Oxidative stress results in a deleterious process that culminates in the damage of cell structures, including membranes and lipids, as well as proteins and DNA [7]. Reactive oxygen species (ROS) are constantly produced by enzymatic and non-enzymatic reactions. The major

reactions catalysed by the action of `enzymes that generate ROS include those involving NADPH oxidase, nitric oxide synthase (NOS), xanthine oxidase, arachidonic acid, and metabolic enzymes such as the cytochrome P450 enzymes, cyclooxygenase, and lipoxygenase. The non-enzymatic production of ROS comes from the mitochondrial respiratory chain. The major ROS produced in the human organism include singlet oxygen (1O2), superoxide anion (O2), hydroxyl radical (OH,), hydrogen peroxide (H2O2) and organic peroxides [8]. In addition, other molecules that affect oxidative balance are the reactive nitrogen species (RNS), such as nitric oxide.

Oxidative imbalance is chargeable for generating numerous reactive molecules, which might be scavenged through Orz or its metabolites. The intake of high-fats diets (HFD) has been proven to result in the formation of unfastened radicals and ROS, ensuing in lipid peroxidation and oxidative stress [24]. Orz and FA suppressed lipid peroxidation in mice fed a HFD intake primarily based totally on an eating regimen that covered 15 mg/day of each compounds decreased plasma and erythrocyte thiobarbituric acid reactive substances (TBARS), while in comparison to manipulated mice fed the HFD alone. This locating illustrates that Orz and FA can act as ROS scavengers and save you lipid peroxidation. Furthermore, the prevention of lipid peroxidation avoids lipotoxicity, that is related to mitochondrial dysfunction, and formation of cell ROS. In addition, those compounds have the ability to lessen glucose-6-phosphate dehydrogenase (G6PD) [9], which promotes the expression of pro-oxidative enzymes NAPDH oxidase and NOS. Metabolites of Orz can result in exceptional antioxidant responses withinside the organism, as discovered inside the serum tiers of overall antioxidant ability (TAOC) and malondialdehyde (MDA) in rats. In serum, decreased TAOC and improved MDA content material turned into precipitated through HFD. However, FA treatment better improved TAOC and MDA levels when compared to Orz [9, 10, 11,12].

The number of people over the age of 60 on the world stage is projected to increase twice as fast as in the past four years. In terms of longer life expectancy, human body cells are more prone to chronic diseases such as diabetes, heart disease, and cancer due to lack of essential nutrients. As the condition is reaching an epidemic in many parts of the world, nutritional diet is demonstrated as the most important non-genetic contributor to human health. [1,2] In fact, components present in many naturally occurring foods have been recognized to play important roles as promoters or

inhibitors of these diseases. Thus, these phytochemicals present in their natural form may help as a major group component with beneficial effects in preventing or delaying the onset of nutritional diseases in specific population groups. Knowing how these components affect the health of human life will be possible only when we get a better understanding of the molecular basis of these components. [3] In this process the following areas of research are attainment special significance:

Application of omics technologies such as nutrimetabolomics, nutriproteomics and nutrigenomics to gain a deeper understanding of the role of these dietary components for the biological processes occurring in the cells of the human body.
Human behavior and environment influence the way your genes work, ie analyzing the effects of dietary components and their relationship to human health through epigenetics.
Identification and validation of biomarkers to monitor the effects of dietary components on chronic diseases.

Based on existing molecular scientific evidence study the clinical involvement readings in explicit population groups.

Cancer as a genetic disorder is the main and first cause of death in economically developed countries and second in emerging countries. [4] Various reports indicate that around 13% of the total deaths globally i.e. 7.6 million pearls are caused by cancer and its global burden is largely due to the growth of the world's population and aging as well as inducing cancer. The behavior has increased especially in relation to increased smoking intake. [5, 6] Most of these disorders result from colon, lung, colon, breast and liver cancers. [7,8] Through various research and studies, it has been estimated that in the treatment of approximately 50% - 60% of cancer patients in the United States, the plant or its nutrients as a supplementary agent and/or substitute medicine, either completely or conventionally. Used as a drug with medical treatment such as chemotherapy and /or radiation therapy. [9, 10]

For thousands of years, various naturally occurring plant parts have been used as herbs for the prevention and/or treatment of a variety of diseases. [11] The presence of various bioactive components present in plants makes them a particularly suitable choice for use by gourmet food consumers. [12-14] Based on the evidence obtained from various experiments, it has been

confirmed that the bioactive components obtained from natural plants have anti-cancer activities and these bioactive components can be used naturally for the prevention and/or treatment of cancer. [15-19] A great deal of research has been devoted to dietary phytochemicals that have had a constructive effect on human health, resulting in an increased understanding of these phytochemicals, or potentially helpful compounds found in plant foods, as biological and chemically functional agents. Their use involving dietary phytochemicals has been explained with the advent of in vitro i.e. molecular, genomic, and various cellular testing systems, and the development of various studies conducted through in vivo i.e. transgenic and knockout animal models.

METERIALS AND METHODS

Gamma Oryzanol:-

Gamma original was previously treated as a single ingredient in rice bran oil. [20] But it was later determined that gamma is a fraction of the original plant sterols containing the ferrate (4-hydroxy-3-methoxy cinnamic acid) ester and triterpene alcohols. About 80 percent of Gamma Original is found in the form of three major constituents which chemically include 24-methylenecycloartanyl ferulate, cycloartenyl ferulate, and campesteryl ferulate (Figure 1). The constituents of gamma origin present in rice bran oil can be quantified by high performance liquid chromatography. [21]

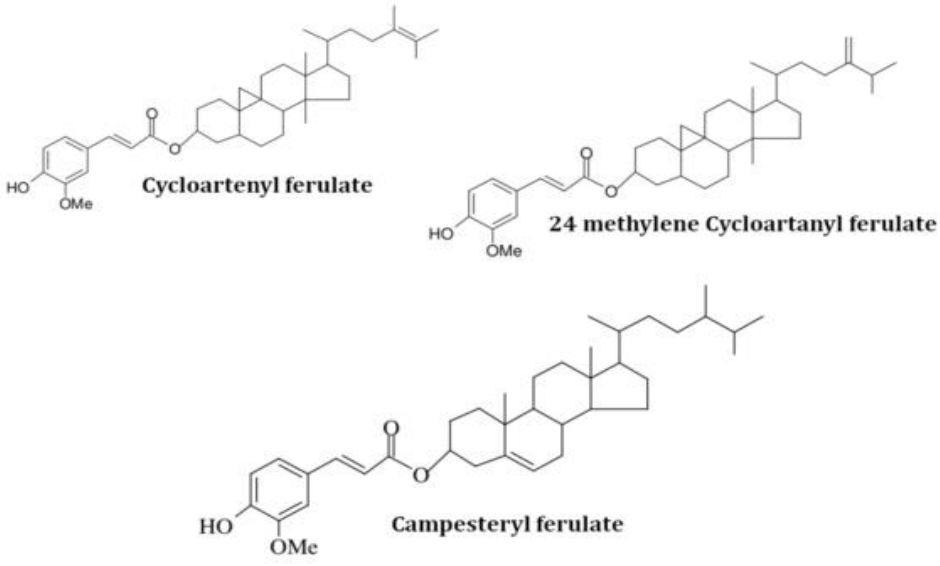


Figure 1:- Fraction of Rice Brain Oil

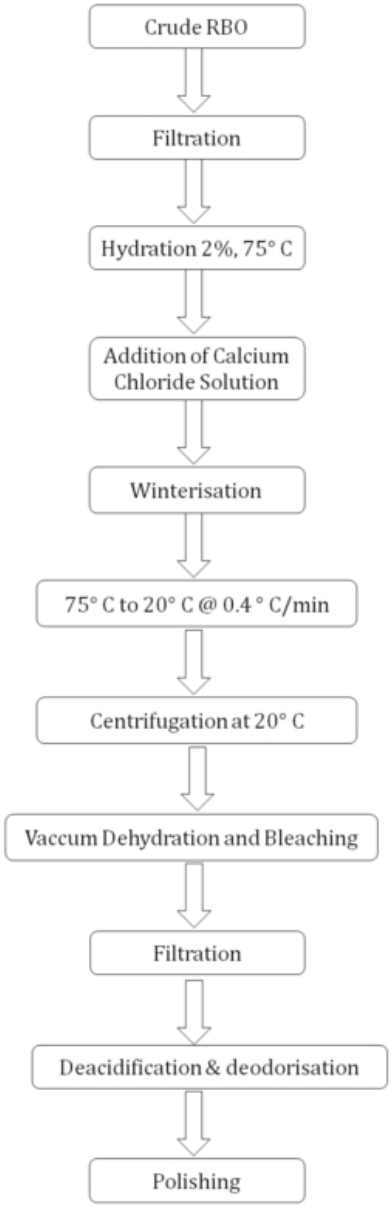
Extraction and Refining of Rice Bran Oil

Hexane is used as a solvent to chemically extract the oil from rice bran, although hexane is potentially harmful to animal health and the environment. Given the potential for health and environmental hazards of hexane, short-chain alcohols, especially ethanol and isopropanol, have been proposed as an alternative extraction solvent due to their greater safety and less potential for regulation. [22-25] There have been many studies using isopropanol as a solvent for the extraction of soybean and cottonseed oil whereas only a few studies have been done on rice bran using isopropanol or ethanol as the extraction solvent. Isopropanol and tocopherols have been used to extract B-vitamin-rich rice bran oil, and ethanol to extract B-vitamin-rich rice bran oil. [26-27] Food-grade rice bran oil is obtained by subjecting crude rice bran oil (CRBO) to physical refining or chemical refining. Gum sludge, wax sludge and soap stock sludge are waste byproducts produced in chemical refining. process and is currently less used. [28]

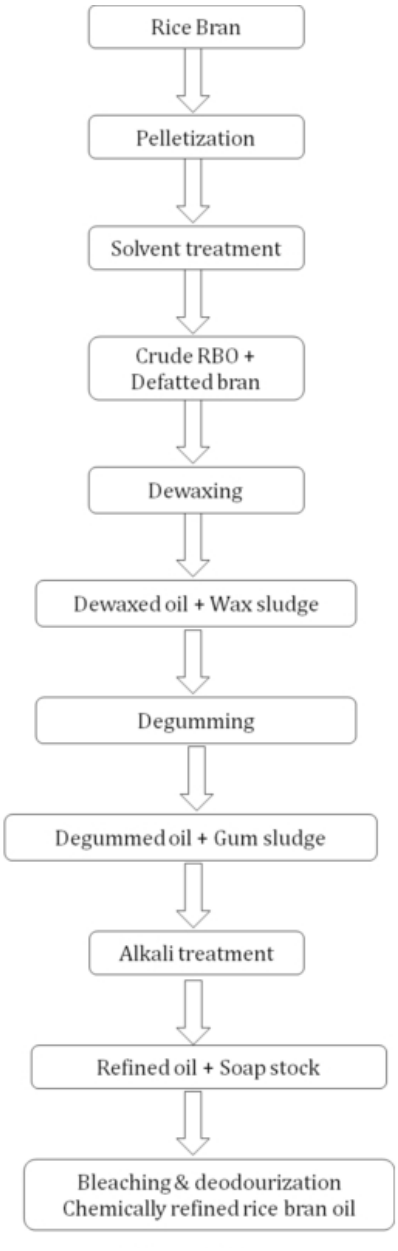
Extraction and Purification of γ-Oryzanol

The removal of gamma-origins present in rice bran can be done using supercritical carbon dioxide techniques or organic solvents. Comparison of solvent extraction and supercritical fluidization method for extracting gamma-originating present in rice bran by Xu and Godbar et al., tested organic solvents. According to the study obtained by the method adopted by him, the production of the highest yield of -oryzanol can be achieved. For this the highest yield of 1.68 mg gamma oryzanol was obtained per gram of rice bran in a solvent mixture with 50% hexane and 50% isopropanol (volume) at 600 °C for 45-60 minutes. [29] The yield of -oryzanol decreases due to saponification during solvent extraction, however, the yield of gamma oryzanol per gram of rice bran in supercritical fluid extraction at 500 °C was found to be 5.39 mg per gram of rice bran at 680 atm pressure solvent extraction for 25 min. was almost four times higher than the highest yield. Additionally, a high concentration of -oryzanol in the extract was obtained by collecting the extract after 15–20 min of extraction under optimized condition ranging from about 50 to -80 percent. Comparative study suggests that -oryzanol can be extracted from crude oil of rice bran and fermentation, corn and barley by distillation of these oils at low temperature. The residue thus obtained is removed with hydrosol solvents and alkalized below 0.5N and the extract is neutralized with organic acids to pH 6. The crude -oryzanol obtained in this process can be crystallized at lon point 135.70 C and UV band

maximum 216,231. [30] Gamma oryzanol can also be extracted by treating rice bran oil with alkali by refluxing the residue with hydrochloric acid and a solution of sodium hydroxide in methanol and hydrolyzing it. While filtering the extract thus obtained, the methanol evaporates off. These residues are treated with dilute hydrochloric acid, dissolved in diethyl ether and treated with disodium trioxide to remove the fatty acids. It is then washed with water and dried and evaporated to give a residue containing oryzanol. [31]



Physical refining of rice bran oil



Chemical refining of rice bran oil

PHARMACOGNOSTICAL EVALUATION

Macroscopic Examination

Colour Untreated samples were examined under diffuse day light. An artificial light source with wavelength similar to those of day light may also be used. The colour of the sample was observed. Surface Characteristics, Texture and Fracture Characteristics Materials was touched to determine if it is soft or hard bend and ruptured it to obtain information on brittleness and the appearance of the fracture plane-whether it is fibrous, smooth, rough, granular, etc.

Odour A small portion of the sample was placed in the palm of the hand and slowly and repeatedly, the air was inhaled over the material. Taste A small amount of drug powder was kept over the tongue and the taste was observed. Loss on Drying For estimation of loss on drying, it was dried at 105°C for 5 hours in a hot air oven, cooled in a desiccator for 30 minutes, and weighed without delay. The loss of weight was calculated as the content of in mg per g of air-dried material.

Microscopic Examination: Microscopic examinations of medicinal plants are not only essential to the study of the adulterants but also are indispensable in the correct identification. Classification of Microscopic Particles is according to the presence of cell contents, such as starch grains, aleurone grains, plastids, fats and oils may render sections non-translucent and obscure certain microscopic characters. Many reagents that dissolve some of these contents were used in order to make the remaining parts stand out clearly or produce a penetrating effect. This has been rendered the section more transparent revealed details of the structures. A solution of 10% potassium hydroxide was used as a clarifying agent. The phloroglucinol and hydrochloric acid were placed on one edge of coverslip of a prepared specimen slide. The excessive fluid under the cover slip of the slide was removed by using a strip of tissue paper. The different cell contents were observed by following methods.

Quantitative Microscopy The fresh oil was subjected to standard procedure for the determination of various leaf constants. Determination of Stomatal Number Leaf fragments of 5x5 mm approx. in size were placed in test tube containing 5ml of chloral hydrate solution. This was heated in a boiling water bath for about 15 minutes or until the fragments became transparent. Fragments were then transferred to a microscopic slide and the mount was prepared

with the lower epidermis uppermost, in chloral hydrate solution. Afterward a drop of glycerol-ethanol solution was put on one side of the cover glass to prevent the preparation from drying. It was examined with a 40X objective and with 6X eyepiece, to which a microscopical drawing apparatus is attached. Mark on the drawing paper a cross (X) for each stomata. Thus, for each surface of the leaf, average number of stomata per square millimeter was calculated.

Physicochemical Evaluation Determination of Foreign Matter about 10 gm of sample has been weighed and spread on a white tile uniformly, without overlapping. Then the sample was inspected by means of 5X lens and the foreign organic matter has been separated. After complete separation, the matter was weighed and percentage w/w was determined.

Determination of Solvent Extractive Value

Determination of Water-Soluble Extractive Value Five gm of powdered drug was macerated with 100 ml of water closed flask for 2hr and was occasionally shakes with 6hr time period and was allowed to stand for 18hr. After filtration the 25ml of the filtrate evaporated to dryness in a tarred flat-bottomed shallow dish. Dried at 105°C and weighed. Percentage of water-soluble extractive value was calculated with reference to the air-dried drug.

Determination of Ethanol-Soluble Extractive Value

Ethanol is an ideal solvent for extraction of various chemicals like tannins, alkaloids, resins etc. Ethanol (95% V/V) was used for determination of ethanol soluble extractive. Five gm of powdered drug was macerated with 100ml of ethanol closed flask for 24 hours and was occasionally shakes with 6 hours' time period and was allowed to stand for 18 hours. After filtration the 25 ml of the filtrate evaporated to dryness in a tarred flat bottomed shallow dish. Dried at 105°C and weighed. Percentage of ethanol soluble extractive value was calculated with reference to the air-dried drug.

Determination of Moisture Content

The percentage of active constituents in crude drugs is mentioned on air-dried bases. Hence, the moisture content of the crude drugs should be determined and should also be controlled. The moisture content should be minimized in order to prevent decomposition of crude drugs either due to chemical changes or microbial contamination.

Procedure: The powdered sample of leaves of oil weighed 5gm accurately and kept in IR moisture balance. The loss in weight was recorded as a a percentage (%) moisture with respect to air-dried sample of crude drug.

Determination of Ash Value

The residue remaining after incineration is the ash content of the drug, which simply represents inorganic salts, naturally occurring in drugs or adhering to it or deliberately added to it as a form of adulteration. Many a time the crude drugs are mixed with various mineral substances like sand, soil, calcium oxalate, chalk powder, or other drugs with different inorganic content. Ash value is a creation to judge the purity of crude drugs. Generally, either ash value or acid-insoluble ash value or both is determined.

Total ash usually consists of phosphates, silicates, and silica. On the other hand, acid-insoluble ash, which is a part of total ash insoluble in dilute hydrochloric acid, contains adhering dirt and sand.

Determination of Foreign Matter

Weigh 100-500 gm of the drug sample to be examined or the maximum quantity prescribed in the monograph, and spread it out in a thin layer. The foreign matter should be totally free from insect, moulds, and harmful and poisonous matters and detected by inspection with the unaided eye or by the use of a lens (6X). Separate and weigh it and calculate the amount and percentage present.

Preliminary Phytochemical Evaluation of Extraction of oil-

The extracts obtained were subjected to various qualitative tests to reveal the presence or absence of common phytopharmaceuticals. The extracts obtained were subjected to various qualitative tests to reveal the presence or absence of common phytopharmaceuticals.

a. Alkaloids Small portion of alcoholic extract was stirred separately with a few drops of dilute hydrochloric acid and then filtered. The filtrate is then tested carefully with various alkaloid reagents such as: Mayer’s Reagents Alkaloids give precipitate with Mayer’s reagents. One ml of

Mayer's reagent (Potassium mercuric iodide solution) was added to 1 ml extract, whitish yellow or cream-colored precipitate indicated the presence of alkaloids.

b. Dragendroff's Reagents Alkaloids give orange brown precipitate with Dragendorff's reagents. One ml of Dragendorff's reagent (Potassium bismuth iodide solution) was added to 1 ml extract, an orange-red precipitate indicated the presence of alkaloids. Hager's Reagents Alkaloids give yellow coloured precipitate with Hager's reagents. In to the 1 ml extract, 3 ml of Hager's reagent (saturated aqueous solution of picric acid) was added, a yellow coloured precipitate indicated the presence of alkaloids. Wagner's Reagents Alkaloids give reddish brown precipitate with Wagner's reagents. In to 1 ml extract, 2 ml of Wagner's reagent (iodine in potassium iodide) was added and the formation of a reddish-brown precipitate indicated the presence of alkaloids.

c. Carbohydrates and Glycosides A small quantity of each extract dissolved separately in distilled water and was filtered. The filtrate is subjected to the following test for Carbohydrates. Molisch's Test One ml of α-naphthol solution and concentrated sulphuric acid was added in 2 ml of the extract, through the side of the test tube. The formation of purple or reddish violet color at the junction of the two liquids reveals the presence of carbohydrates. Fehling's Solution Equal volume of Fehling's A (copper sulfate in distilled water) and Fehling's B (potassium tartrate and sodium hydroxide in distilled water) reagent was mixed along with few drops of extract solution and boiled, a brick red precipitate of cuprous oxide forms. Benedict's test Extract solution was treated with few drops of Benedict reagent (alkaline Solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate forms, if reducing sugar is present.

d. Tollen's test To 100 mg of compound add 2ml of Tollen's reagent and heat gently; a silver mirror is obtained inside the wall of the test tube. It indicates the presence of aldose sugar. Keddes reagent test Cardenolides give blue or violet color with this reagent which fads after 1-2 hour. This reagent is prepared by mixing equal volume of 0.2% solution of 3, 5 dinitro benzoic acid in 100 ml of 0.5 N KOH solution in 50% methanol.

e. Legal's test Treat the extract with pyridine and add alkaline sodium nitroprusside solution, blood red color appears.

f. Keller Killiani Test 1gm of powdered drug extracted with 10ml of 70% alcohol for few minutes and filtered. To 5 ml of this filtrate 10 ml of hydrogen peroxide solution and 0.5 ml of strong solution of lead acetate is added. Precipitate thus obtained is filtered. Filtrate is shaken with 5 ml of chloroform layer is separated and to this 1ml of mixture of 1 volume of 5% ferric sulfated and 99 volume of glacial acetic acid is added. To this mixture 1 or 2 drops of concentrated sulfuric acid is added. Appearance of blue color confirms the presence of deoxy sugars.

Acute Toxicity Study of Extract (LD50)

Acute oral toxicity studies have been conducted on an individual basis followed by using OECD guideline 423. The method used defined doses of 5, 50, 300, 2000 mg/kg p.o. body weight. Results were allowed substance rank and classify according to the Globally Harmonized System (GHS) for classification of chemicals which cause acute toxicity. From LD50 determination, 1/10th of the dose was focused as the medial for pharmacological screening. Since all the animals were alive; no mortality, no toxicity and no significant changes in the body weight between the control and treated group were observed at a dose of 2000 mg for 72 hours. This finding probably suggests that the ethanol and aqueous extract are relatively safe or non-toxic in rats at the doses used for this study. The present study has been carried out to evaluate the LD50 . All drugs have been obtained from Pallav Chemicals Pvt. Ltd., Bombay. All extracts were suspended with the help of gum acacia in distilled water at the time of oral administration.

ANTIOXIDENT ACTIVITY

Preparation of rice bran powders of two varieties of Iranian rice, namely Fajr and Tarem, were obtained by milling rice grain in a local grinding mill in Babolsar, followed by sieving to separate grain from rice bran. Stabilization of rice bran was carried out in a microwave oven with 550 W output power. One hundred grams of each sample was packed in a polyethylene microwave-safe bag and subjected to microwave heating in a preheated oven for 3 min at 120 °C, and then cooled down at room temperature overnight. This procedure was repeated three times to ensure the stabilization. Then the samples were placed in the cooler at 4 °C for one week until analyses [32].

Extraction of total antioxidants

Five grams of stabilized rice bran were extracted with 20 ml of methanol (MeOH), ethyl acetate (EtOAc), and ethanol (EtOH) at room temperature for 3 h in an electrical shaker. The residue was re-extracted twice and filtered through the Whatman No. 1 filter paper. The extracts were combined and dried under vacuum, using a rotary evaporator (50 °C), and weighed immediately.

Determination of total phenolic content The total phenolic content of bran extracts was determined, using the Folin–Ciocalteu reagent [33]. The reaction mixture contained 20 µL of bran extracts mixed with 100 µL of the freshly prepared Folin–Ciocalteu reagent and a further 1.58 ml of distilled water. The mixture was shaken vigorously and 300 µL of sodium carbonate (20% w/v) was added and the mixture was again shaken for 2 min. After the mixture was left to stand for 2 h at room temperature, the absorbance at 765 nm was measured by using a UV-vis spectrophotometer. Gallic acid was used as a standard, and results were calculated as Gallic acid equivalents (mg/g of bran).

Scavenging effect on DPPH radical Free radical scavenging activities of bran extracts was determined by using a stable DPPH radical [34]. 0.1 ml of the extract solution was well mixed with 3.9 ml of methanol and 1.0 ml of DPPH solution. The mixture was kept at ambient temperature for 30 min before measurement of the absorbance at 517 nm. The scavenging effect was derived following Eq. (1):

$$\text{DPPH scavenging \%} = [1/(A517\ \text{nm, sample} - A517\ \text{nm, control})] \times 100. \quad (1)$$

Determination of reducing power Extracts (10, 25, 50 mg/ml) was mixed with Phosphate Buffer (2.5 ml, 2.0 M, pH 6.6). The dilute sample was then mixed with 2.5 ml of 1% potassium ferricyanide and incubated at 50 °C for 20 min. About 2.5 ml of 10% trichloroacetic acid were added and the mixture was centrifuged for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and 1% ferric chloride (0.5 ml), and absorbance was measured at 700 nm [35].

Inhibition of lipid peroxidation (Ferric-Thiocyanate Method) 100 µL of linoleic acid was dissolved in 4 ml of EtOH, 8 ml of 0.05 M phosphate buffer (pH 7.0) and 3.9 ml of distilled water. 50 µL of sample (10, 25, 50 mg/ml) was added to 1.4 mL of the previously described

linoleic acid solution. This mixture was kept in darkness and at 40 °C; the accelerated oxidation of linoleic acid was measured after 24, 48, 72 and 96 h of thermal treatment. The determination of oxidation degree (as peroxides formation) was performed according to the ferricthiocyanate method: 30 µL of the reaction mixture was added to 2.91 ml of 75% ethanol, 30 µL of 30% ammonium thiocyanate and 30 µL of 0.02 M ferrous chloride in 3.5% hydrochloric acid. Mixtures were shaken and exactly after 3 min the absorbance was measured at 500 nm [36. The percentage of LA peroxidation inhibition was calculated by Eq. (2):

Inhibition on LA peroxidation % = [1/(A500 nm, sample − A500 nm, control)] × 100. (2)

Statistical analysis

In this study, Media optimization for antioxidant activity was carried out by Response Surface Methodology (RSM). The Central Composite Design (CCD) was used [37]. It allows the determination of both linear and quadratic models. In general, a CCD for k factors, coded as (x1, . . . , xk), consists of three parts: a factorial (or cubic) design containing a total of fact = 2 k points with coordinates xi = −1 or xi = +1, for i = 1, . . . , k; an axial (or star) part formed by nax = 2k points with all their coordinates null, except for the one that is set equal to a certain value α (or - α), which usually ranges from 1 to √ k; and finally a total of nc runs performed at the center point of the experimental region, where, of course, x1 = x2 = · · · = xk = 0. A p-value of < 0.05 was considered statistically significant.

Pharmacognostical Evaluation

Macroscopic Examination The macroscopic examination of the plant Streblus asper was carried out. The results are reported in table.

Table 1: Morphological Parameters of rice bran

s.n	parameter	result
1	Size	8-10 cm
2	Shape	Cylindrical
3	Test	Testless
4	order	Oderless

Micromeritic Evaluation The air-dried powder of the gamma organal rice bran was evaluated. The results are reported in Table 2.

Table 2: Micromeritic Parameters of rice bran.

s.n	powder	parameters
1	Angel repose	0.32
2	Bulk density	1.37gm/m
3	Tapped density	193gm/m

Physicochemical Evaluation

The physicochemical evaluation of the plant Streblus asper L. (Leaves) was carried out. Air dried material was used for the quantitative determination of physiochemical values in this study ash values (total ash, acid insoluble ash and water-soluble ash), moisture content, swelling index and foreign organic matters were determined. Alcohol and water soluble extractives were determined and were recorded. Alcohol and water extractive were determined as per WHO recommendations. Water soluble extractive was found to be very high when compared to other extractable matter in the drug. The results are reported in table 3.

Table 3: Physiochemical parameters of rice bran

s.n	Parameter	Rice bran
1	Loss of drying	5.6
2	Total ash	3.5
3	Acid insoluble ash	0.96
4	Water insoluble ash	1.63
5	Aqueous extractive value	12.5
6	Ethanloc extractive value	13.8

Preliminary Phytochemical Screening of rice bran

The extract obtained after extraction of the gamma rice bran oil was subject to phytochemical screening which revealed the present of various active phytoconstituents. The results were presented in (table 4).

Table 4: Preliminary Phytochemical Screenings of rice bran

s.n	Chemical constituent	ethanol	methanol
1	Alkaloid	+	+
2	Carbohydrate	+	+
3	Glycoside	+	-
4	Steroid	-	-
5	Saponin	+	+
6	Amino acid	-	+
7	protein	+	+

Acute Toxicity Study of Extract (LD50) The aqueous and ethanolic extracts the plant material viz rice bran was screened for acute toxicity study by OECD guideline no. 423 for determination of LD50. The result indicates 200 mg/kg dose has been considered as effective dose (ED50).

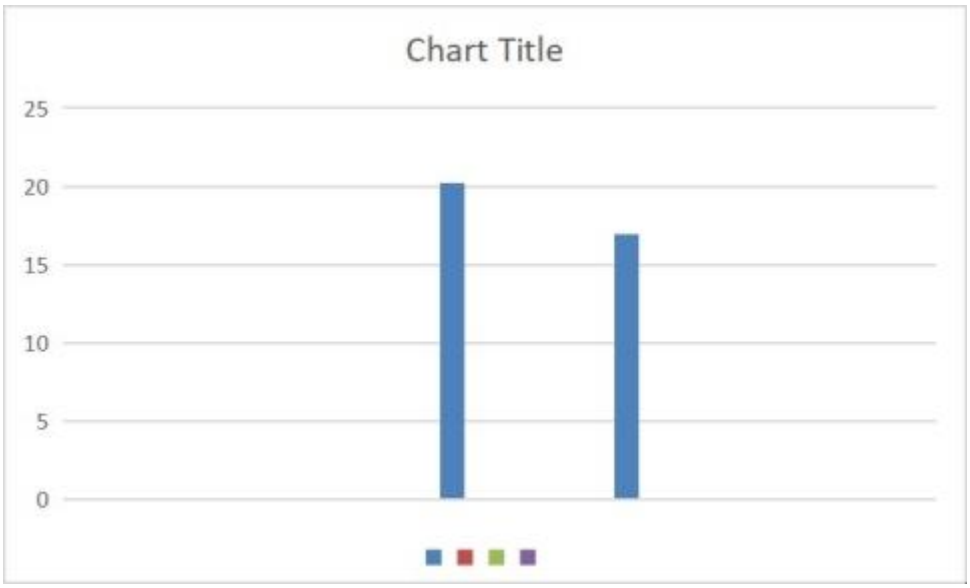
Extract	LD50	ED50
RICE BRAN OIL	2000mg/kg	300mg/kg

Pharmacological Screening of Extract

ANTIOXIDANT AC TIVITY

Table 5: Yields and total phenolic content of rice bran with solvents.

s.n	**solvent**	**Extract yield**	**Total phenolic content (mg Gallic acid/g of bran)**
1	Methanol	20.179	3.31 ± 0.04
2	ethanol	16.95	1.39 ± 0.04



CONCLUSION

The present work carries the results of 'Pharmacognostical and Pharmacological evaluation of rice bran. It indicates the utilization of plants for the treatment of various ailments among the inhabitants along and it was also validated scientifically by pharmacological screening. At the various conc. of drug extract show the pharmacological activities used in the traditional system have to show the various medicinal properties.

RECOMMENDATION

Extraction of antioxidant phytocompounds from Fajr rice bran with MeOH produced a significantly greater yield and total content in phenolic compounds than ethanol and ethyl acetate solvents, which lead to high antioxidant activities of extracts in inhibiting linoleic acid peroxidation, DPPH radical scavenging ability and reducing power. The strong antioxidative activity of rice bran extracts might be due to the presence of main tocotrienols or the synergistic effect of tocopherols and tocotrienols.

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