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Anti-oxidant Activity and Biochemical Evaluation of Rice Bran

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KEYWORDS

Oryza Sativa, gama-oryzanol, antioxidant, Phytochemicals, DPPH,.

ABSTRACT:

Introduction: Rice is very commonly used food product all over the word, Asia, China, India and other country. It is used as food from ancient time. Rice bran obtained from milling of rice traditionally used as a medicinal product for treatment of disease and journal purpose. Rice bran obtained from various rice verities are used for treatment of various disease and cosmetic product. Several study stated that the chemical present in rice bran is used for treatment of various disease like diabetes, cancer, heart disease and beautifying agent etc. chemical present in rice bran like tocol and oryzanol, Phytosterol, Tocotrienol, fiber and Oryzanol help in lowering cholesterol, anti-cancerous and cardioprotective effect. Extract of bran exhibit antioxidant property due to presence of phytochemicals phenols, pigments, oryzanol and tocols and anti-carcinogenic property due to presence of hemicelluloses and Tocotrienol.

Objectives: The objective of this study was to investigate the phytochemical present on rice bran by various chemical test of bran extract and in-vitro study of antioxidant activity. Anti oxidant activity study by using DPPH (2'2-Diphenyl-1-picrylhydrazyl) assay method and Nitric oxide scavenging activity method

Methods: Study involved step by step process and various method to determined its phytochemicals and antioxidant property. extract was obtained by using soxhlet extraction process. These extract was used for phytochemical screening, identification of the various organic compound, inorganic compound and other active constituents like alkaloid, Flavonoide, Glycosides ,Steroid, Protein and Carbohydrate. Free radicle scavenging activity determined by the help of DPPH (2'2-Diphenyl-1-picrylhydrazyl) assay method and Nitric oxide scavenging activity method.

Results: The study of various phytochemical was done by soxhlet extraction process. the obtained extract of rice bran from petroleum ether, ethanol solvent used for the study. Extractive value of prtrolium ether and ethanol was found to be 4.8% and 9.7 % respectively. The phytochemical screening show the presence of alkaloid, Flavonoide, Glycosides ,Steroid, Protein and Carbohydrate and inorganic constituents like calcium, magnesium, potassium iron and absence of phosphate, chloride, sulphate. DPPH and nitric oxide scavenging activity based on the concentration of the rice bran extract. Ethanolic fraction of rice bran useful for reducing free radicle related cell damage..

Conclusions: Rice bran contains significant medicinal value due to presence of various bioactive compounds like glycoside flavonoide alkaloid etc.. Hydro-alcoholic extract of Rice bran use to reveals that the extract contain various bioactive compound and exhibit significant antioxidant activity. Further in-vivo study of extract help to better understanding of the traditional clam of *Oryza sativa* as a medicinal plant.

1. Introduction

Rice is common food for many countries in Asia and all over word. Rice grain contains outer covering husk, bran and embryo. After removal of husk/outer covering whole rice is called kernel. Further removal of bran remaining rice is known as polish rice.[1] Rice bran belonging to family Poaceae also known as *Graminae.*

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More and more Polishing of rice produce lightening of grain. Now a day most of population move toward the natural system of treatment in sted of modern system of treatment. There are various plant species containing medicinal activity has been discovered. The importance of this plant has been increased due to fewer side effects as compared to the synthetic drug and various micro-organism developed resistances against to these synthetic drugs. Hence Rice bran used as traditional medicine in various countries for reliving various diseases like heart disease, cancer, hypercholesterolemia, diabetes etc. [2,3,4] Rice bran contributes approximately 8% part of whole grain. Rice bran is rich source of vitamins, minerals like calcium, potassium, phosphorus, oil wax, trace element, phytosterol, antioxidant compound and other phytochemical [5,6]. Rice bran mote only rich source of nutritional supplement to prevent nutrition diffiencly and malnutrition in children but also rich source of nutraceuticals for controlling Varity of body dis-functioning like hypertension, dyslipidemia, arthritis, diabetes [7,8,9]. It is also reported that rice bran help to treat the coronary heart disease, arthrosclerosis. Rice bran reduce the body body cholesterol level due to presence of its laxative property[10].

In Chhattisgarh, India traditionally rice bran used as medicinal value [11]. Laicha is skin infection which treated by Laicha Rice Varity. Anciently it also used as animal feed, making bags mates etc. now rice bran oil extracted from rice bran have high medicinal value. Traditionally verity of rice used for treatment of various diseases like lowering blood glucose level, health benefits, improve men's fertility, prevention of cancer [12]. Rice bran contain superior quality of protein because its protein efficiency ratio (PER) is high, hypo-allergic property and lysine content [13]. Rice bran contain bioactive potential due to presence of cellulose, fiber, oil like tocol and oryzanol etc like bioactive compound. Phytosterol, Tocotrienol, fiber and Oryzanol help in lowering cholesterol, anti-cancerous and cardioprotective effect [14,15,16,17]. Extract of bran exhibit antioxidant property due to presence of phytochemicals phenols, pigments, oryzanol and tocols [18,19], And anti carcinogenic property due to presence of hemicelluloses and Tocotrienol [20,21] .

2. Objectives

The present study based on the rice bran belonging to the family *Graminae.* Which focus on the anti-oxidant activity of rice bran. Antioxidant activity of rice bran helps to protect cell oxidation by free radicle. This study was done by DPPH assay method and Nitric oxide scavenging activity method, and successive investigation of their various chemicals.

3. Materials And Methods

Collection and authentication

Fresh rice bran was collected from Rice mill plant of Mau district, Uttar Pradesh and Rice bran extract Obtained from solvent extraction process. The plant material (Rice Bran) was authenticated by Birbal Sahni Institute of Palaeosciences, Lucknow (U.P.).

Phytochemical Investigations

Extraction (soxhlet extraction)

The rice bran was used for extraction process. About 2.5 kg of coarsely powdered of rice bran was subjected to successive extraction in a soxhlet apparatus with various solvents of increasing polarity (petroleum ether, ethanol). The individual extract was filtered, concentrated under rotary vacuum evaporator following on the boiling water bath to obtain sticky solid mass which was further dried in lyophilizer. The percentage yield of various extracts was calculated by the given formula. The percentage yield shown in Table No. 1.

$$\% \text{ yield} = \frac{\text{Amount of Extract}}{\text{Amount of crude drug}} \times 100$$

Preliminary Phytochemical Screening

Test of Organic Elements [22]

a) Tests for Carbohydrates

A sufficient amount of the extract was dissolved separately in ethanol and filtered. The filtrate was subjected to the various chemical tests to detect the presence of various phyto-constituents.

***Molish's test (general test)*:** The filtrate was treated with 2-3 drops of molish reagent (α naphthol in 1%

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alcohol) and the concentrated H2SO4 was added along the sides of the test tube. Appearance of violet ring may form at the junction of two liquids which may show the presence of carbohydrates.

(i)Tests for Reducing Sugars:

***Fehling's test*:** The filtrate was treated with 1 ml of Fehling's solution A and B in equal amount and heated on the water bath (5-10 min). A brick red precipitate may occur to correspond to the presence of carbohydrate.

***Benedict's test*:** The equal volume of filtrate and Benedict's reagent were taken in a test tube then heated on boiling water bath for 5 minute. The solution appeared green, yellow or red which may correspond to the presence of reducing sugars.

b) Tests for Proteins

***Xanthoprotein test (for protein containing tyrosine or tryptophan)*:** 3 ml of test solution with 1 ml of concentrated H2SO4 gave white precipitate. The precipitate when boiled on water bath might give yellow colour precipitate which after addition of NH4OH might turn orange to ensure the presence of protein.

(i)Tests for Amino Acids

***Ninhydrin test (general test)*:** 3 ml of test solution and 3 drops of 5 % ninhydrin solution were mixed in test tube and boiled on water bath for 10 min. purple or bluish colour may indicate the presence of amino acid.

d) Tests for Steroid

***Salkowski reaction*:** 2 ml of test solution was mixed with 2 ml of chloroform and 2 ml of conc. H2SO4 in a test tube and shaken well. The chloroform layer may appear red which indicate the presence of sterols and the acidic layer may show greenish yellow colored fluorescence to reveal the presence of steroid.

***Liebermann - Burchard reaction*:** 2 ml of test solution was mixed with 2 ml of chloroform, 1-2 ml of acetic anhydride and 2 drops of conc. H2SO4 from the side of test tube which may be initially red then blue and finally green colour may appear.

e) Tests for Glycosides

i) Test for Cardiac Glycosides

***Keller-Killiani test (Test for deoxysugars)*:** 2 ml test solution was mixed with glacial acetic acid and added with 1 drop of 5 % FeCl3 followed by addition of conc. H2SO4. Reddish brown colour may appear at the junction of the two liquid layers and upper layer may show bluish green colour which may correspond to the presence of glycosides.

ii) Test for Anthraquinone Glycosides

***Borntrager's test for anthraquinone glycosides*:** 3 ml of extract was boiled with dilute H2SO4, filtered and then cooled. To the cooled filtrate equal volume of benzene or chloroform was added and then shaken well. The organic layer was separated and ammonia was added. The ammoniacl layer may become pink to red to ensure the presence of glycoside.

f) Tests for Flavonoids

***With lead acetate solution*:** The small quantity of residue was taken with lead acetate solution which may show yellow colored precipitate.

g) Tests for Alkaloids

Small quantity of the extract was treated with few drops of diluted hydrochloric acid mixed well and filtered. The filtrate was used for the following tests.

***Mayer's test (Potassium mercuric iodide solution)*:** 2-3 ml of filtrate was mixed with few drops of Mayer's reagent; cream coloured precipitate may produced to indicate the presence of alkaloids.

***Dragendroff's test (Potassium bismuth iodide solution)*:** 2-3 ml of filtrate and few drops of Dragendroff's reagent were mixed in a test tube. A reddish brown precipitate may indicate the presence of alkaloids.

Tests for Inorganic Elements [22]

Ash of drug material was prepared and to this 50% v/v HCl or 50% v/v HNO3 was added. It was kept for 1 hour or longer and then filtered. With the filtrate, following tests were performed.

1) Test for Calcium:

10 ml of filtrate was mixed with 1 drop of NH4OH and saturated ammonium oxalate solution. White precipitate of calcium oxalate may be soluble in HCl but insoluble in acetic acid.

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2) Test for Magnesium:

Calcium oxalate precipitate was filtered and separated from the above procedure. The filtrate was heated and then cooled with solution of sodium phosphate in dilute ammonia solution. White crystalline precipitate may indicate the presence of Mg.

3) Test for Potassium:

2-3 ml of test solution and few drops of cobalt nitrite solution were taken in a test tube. Yellow precipitate of potassium cobalt nitrite may appear.

4) Test for Iron:

a) 5 ml of test solution and few drops of potassium ferrocyanide were taken in a test tube which may show dark blue colour.

b) 5 ml of test solution and few drops of 5% of ammonium thiocyanate (or 5% potassium thiocyanate solution) were mixed in a test tube. Blood red colour may appear.

5) Test for Sulphate

a) 5 ml of test solution was mixed with few drops of 5% BaCl2. White crystalline precipitate may indicate the presence of sulphate.

b) With the lead acetate reagent given white precipitate and then the precipitate may be soluble in NaOH.

6) Test for Phosphate

5 ml of test solution was mixed with HNO3 and few drops of ammonium molybdate solution, then heated for 10 min. and cooled. A yellow crystalline precipitate of ammonium phosphomolybdate may indicate the presence of phosphate.

7) Test for Chloride:

a) 3 ml test solution mixed with HNO3 and few drops of 10% AgNO3 solution was added. White precipitate of AgCl may appear which might be soluble in dilute ammonia.

b) 5 to 7 ml of filtrate was added with 3 to 5 ml of lead acetate solution. White precipitates thus obtained may be soluble in hot water which might show the presence of chloride.

Extraction of rice bran oil from Soxhlet apparatus [23]

100 grams prepared rice bran were weighed and filled into a soxhlet extractor. Approximately, 300 mL of *n*-hexane were added to the extraction flask, which were connected to the extractor and condenser. After extraction begin, the solvent flow rate adjusted manually up to 7 cycles per min. Finally, the extraction process was terminated after 100 cycles. After extraction was complete, *n*-hexane was removed under reduced pressure at 50 °C by using a rotary evaporator. The flasks were placed in a desiccator chamber for 1 hour. Finally extract obtained by soxlet extraction process was weighed and the yield was calculated.

ANTI-OXIDANT ACTIVITY

DPPH (2'2-Diphenyl-1-picrylhydrazyl) assay [24] Free radical scavenging activity of ethanolic extract of rice bran can be determined by DPPH assay method. The reaction mixture of dilution series (10,20,30,40, 50,60,70, 80,90,100 mg/ml) taking different volumes of extract will be incubated with DPPH (3ml; 0.15 mM) solutions in methanol. The solution will be allowed to stand for 30 minutes at room temperature. Extracts when reacted with DPPH, a stable purple colored free radical will convert into colorless compound α-α diphenyl β-picryl hydrazine will be formed. The extent of decoloration indicates the amount of DPPH scavenged. The absorbance will be measured at 517 nm. Ascorbic acid will be used as a reference antioxidant. The percent inhibition of DPPH will be calculated using the formula:

$$\% \text{ inhibition of DPPH} = \frac{\text{Absorbance of control}\mathbf{(C)} - \text{absorbance of test sample}\mathbf{(T)}}{\text{Absorbance of control}\mathbf{(C)}} \times 100$$

C is the absorbance of control and **T** is the test sample.

Nitric oxide scavenging activity [24]

ethanolic extract of rice bran (3 mL) of different dilution series (10,20,30,40,50,60,70,80, 90,100 µg/mL) was transferred to the test tubes. Thereafter, 2 mL of the reaction mixture [1.0 mM sodium nitroprusside (SNP) in 0.5 M phosphate buffer, pH 7.4] were added and mixed well. The mixture was incubated for 60 min at 37°C. After incubation, Griess reagent (0.1% *α*-naphthyl-ethylenediamine in water and 1% H2SO4 in 5% H3PO4) was added to the mixtures. The absorbance of the samples was measured spectrophotometrically, at

$$\% \text{ NO scavenging activity} = \frac{\text{Absorbance of control} - \text{absorbance of sample}}{} \times 100$$

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540 nm. ascorbic acid used as a positive control [24]. Nitric oxide (NO) scavenging activity (%) was calculated by, using the formula:

4. Result And Discussion

In this study, various chemical constituents present in extract of rice bran was analyzed by standard screening method. The result of screening method show that the rice bran contain various bioactive chemical like carbohydrate, protein, fat, glycoside etc.

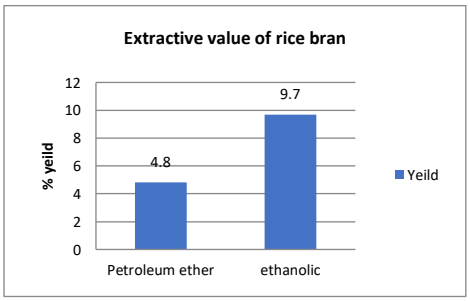
Phytochemical analysis

Extractive value

Extraction of rice bran was performed with various solvent by soxhlet extraction process. The solvent used for extraction of rice bran was petroleum ether, ethanolic solvent and % yield was calculated. Listed in table no-1

Solvent Extract	% Yeld
Petroleum ether	4.8 %
Ethanol	9.7 %

Table- 1: Extractive value of rice bran



Graph-1 Represent % yield of different extractive value

The above graph represent that the ethanolic extract of rice bran show highest % yield as compared to the petroleum ether. The % yield of rice bran extract in ethanolic and petroleum ether extract was found to be 9.7 and 4.8 respectively.

Phytochemical investigation:-

Rice bran extract in various solvent (petroleum ether (PE), ethanolic (E) extract) were used for phytochemical investigation through various chemical test.

Phytochemical analysis of organic constituent:-

The result of Different organic phyto-chemicals constituent analysis was present in rice bran extract shown tin table-2

Test	**PE**	**E**
Carbohydrate		
Molish test	-	-
Fehling's test	-	-
Protein		
Xanthoprotein test	-	+
Ninhydrin test	-	+
Steroid		
Salkowski reaction	-	-
Liebermann-Burchard reaction	-	-
Glycosides		
Keller-Killiani test	+	+
Borntrager's test	+	+
Flavonoids		
lead acetate test	-	+
Alkaloids		
Mayer's test	+	+
Dragendroff's test	+	+

Table -2: organic phyto-constituents of rice bran

Detection of inorganic component:-

Inorganic component of rice bran i.e. zinc, calcium, iron etc were identify by various chemical test and result are given below in table-3

Test	PE	HA
Calcium	+	+
Magnesium	+	+
Potassium	+	+
Iron	+	+
Sulphate	-	-

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Phosphate	-	-
chloride	-	-

Table-3: inorganic component of rice bran

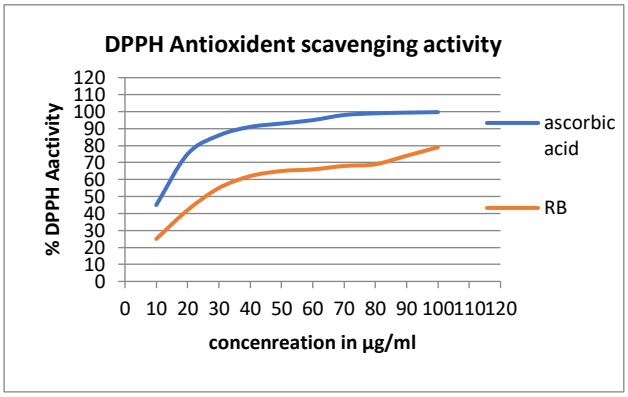
Where (+) Present and (-) absent

Antioxident activity

Antioxidants are those substances that prevent the body damage from free radicle. These free radicle produce during the metabolic decomposition of material in body. These antioxidant molecule help the body from oxidation, which can cause damage of of cell and produce cancer, ageing etc. antioxidants improve our immune system and reduce the risk of cardiovascular disease, cancer, ageing, infection. Antioxidant present in various natural substance like vitamin, and food. Daly taking food containing green vegetables, fruits grains and nuts can supply antioxidant to our body.

Antioxidant activity of rice bran extract identify by using DPPH assay and NO scavenging activity assay method. During this study only absorbance was measured because it is qualitative analysis. Antioxidant activity was measured by using DPPH assay method and Nitric oxide scavenging activity method.

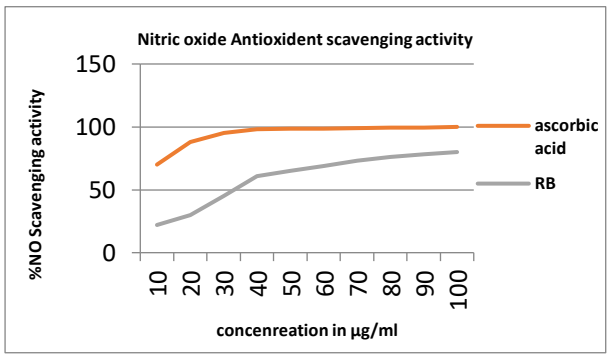
DPPH (2'2-Diphenyl-1-picrylhydrazyl) assay Method: the antioxidant activity of DPPH assay method determined by the discoloration of the solution. In DPPH assay method absorbance was measured at 517 nm wavelengths in UV visible spectrophotometer. DPPH scavenging activity of rice bran based on the transfer of electron from rice bran to the free radicle of the 2'2-Diphenyl-1-picrylhydrazyl molecule. Rice bran extract have strong ability to donate hydrogen ion. This hydrogen donating ability of rice bran decolorized the DPPH. One substance have proton free radicle with distinctive absorption of 2'2-Diphenyl-1-picrylhydrazyl, which is greatly reduce when exposed to the substance that scavenge hydrogen free radicle. It is simplest method[25]**.** The DPPH free radicle scavenging activity of rice bran extract increase with increasing concentration (graph-2). Graph-2 represent the antioxidant activity of rice bran increases as the concentration of rice bran increases. Antioxidant activity of rice bran firstly increases from concentration increases straight line from 0 to 40 µg/ml it become parallel from 40 to 80 µg/ml and again increases straight line from 80 to 100 µg/ml. Hence this graph represents the antioxidant activity of rice bran increases wit increase in concentration.



Graph-2: DPPH Antioxident scavenging activity

Nitric oxide scavenging activity-

Nitric oxide free radicle scavenging activity depend on the concentration of rice bran shown in Graph-3. Nitric oxide scavenging activity of rice bran is compared with ascorbic acid. Nitric oxide scavenging activity of rice bran were lower than the ascorbic acid. As shown in figure 2, rice bran concentration increases from 10 to 100 mg/ml, % nitric oxide scavenging activity increases to free and bound fraction significantly. As concentration increases from 10 to 20 mg/ml % nitric oxide scavenging/antioxidant activity of rice bran increases slowly. When concentration increases from 20 mg/ml to 40 mg/ml % NO scavenging activity increases rapidly and graph become straight line and after that up to 100 mg/ml, % NO scavenging activity increases slowly. Hence this graph represent the % NO scavenging activity of rice bran increase with increasing the concentration of rice bran.



Graph-3: Nitric oxide scavenging activity

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CONCLUSION

Rice bran contains significant medicinal value due to presence of various bioactive compounds like glycoside flavonoide alkaloid etc. rice bran belonging to the family Oryza sativa and used as a traditional medicine for treatment of various disease. Hydro-alcoholic extract of Rice bran use to reveals that the extract contain various bioactive compound and exhibit significant antioxidant activity. Further in-vivo study of extract help to better understanding of the traditional clame of Oryza sativa as a medicinal plant

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