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PHYTOCONSTITUENTS, FREE RADICAL SCAVENGING & ACUTE TOXICITY DETERMINATION OF WHOLE GRAIN AND BROWN RICE

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ABSTRACT

Both Whole grain and Brown rice has been ancient era by human for making various varieties. For completing this research our aim is to find out Phytoconstituents, free radical scavenging activity i.e. DPPH Assay, Hydrogen Peroxide Assay, Total Phenolic content, Total Flavonoid content estimation. After determining these find out acute toxicity of both aqueous extract find that no significant mortality rate was found up to maximum dose 4000 mg/kg was administered.

Keywords: Whole Grain, DPPH, Phenolic, Flavonoid

INTRODUCTION

Beneficial imprudent gratis radicials and oxygen species are highly find in eco natural system. Gratis radicals act on human bodies directly in inner system i.e. nucleic acid, Lipids, Proteins, it can directly generate or cure various disease i.e. cancer, cardiovascular disease, cataracts, diabetes, inflammatory diseases **[1, 3]**. Free radicals are mostly received from plants, it is find in its various shapes like Phytoconstituents, Phenolic components, flavonoids etc. it directly act on oxidative stress which is balanced our body free radicals. Free radicals present and its antioxidant potential shows due to Phenolic acid, flavonones, diterpenines, Quercetin **[4, 5]**. Some scientific research evidences that Phenolic and flavonoids content in plant have possess antineoplastic activities.

Whole grain is an evergreen shrub mostly cultivated in India. Its seeds are large, up to 0.2 cm long and 0.8 cm in diameter contain

seeds in lucid mucilage. Used as food supplement all over the world, it also beneficial in management of diabetes. The traditional uses indicate possible anti-inflammatory and antimicrobial activity **[6, 7]**. It contain other carbohydrates, such as cellulose, hemicelluloses (pentosans), and sugars. Pentosans although their content is low (2-3%) are important owing to their water absorbing capacity i.e. 10 times their mass.

Brown rice is a rich source of various bioactive compounds, such as γ-oryzanol, tocopherol, tocotrienol, amino acids, dietary fibres and minerals. It is less consumed than white rice because its cooking is more difficult than white rice due to its slow water absorption, and the palatability quality of brown rice is inferior to white rice **[8]**.

Evidance of previous result show that whole grain possess antilipidaemic activity, and help in balance body insulin level balance. While brown rice most bioactive compound which possess xanthones and biflavonoids, it exhibit number of pharmacological activities i.e. cytotoxic, antiinflammatory, antifungal.

Aim of design this study to determine antioxidant potential activity and toxicity of whole grain and brown rice. Our work in these to find out phytoconstituent, antioxidant potential and toxicity profile. As per literature and evidence till date no done compilation of this work till date, it is much helpful for determine mentioned research and give best clue to human for its scientific evidence.

MATERIALS AND METHOD

Animals

Albino wistor rats were used for determination of toxicity profile. Albino wistor rats at an average body weight 180-220 grams 3-4 months old was selected for performing study protocol. The animals groups were put in in polypropylene cages and maintained of 23 ± 2ºC. Lighting was synchronized to provide 12 hours of light and 12 hours of dark for every 24 hours. Each cage was renowned by a tag. The animals were fed with standard laboratory animal food pellets with water ad libitum.

Whole Grain

Preparation of Aqueous extract

Maceration :10 gm powdered was placed in a air tight container filled with 1000 ml distilled water. Leaved it for 7 days and gently shaked it. After 7 days extract was filtered out by using whattmann filter paper and dry it **[9]**.

Brown Rice

Preparation of Aqueous extract

Maceration: 10 gm powdered was placed in a air tight container filled with 1000 ml

distilled water. Leaved it for 7 days and gently shaked it. After 7 days extract was filtered out by using whattmann filter paper and dry it **[10]**.

Phytochemical Screening

Phytochemical broadcast of the extracts was conceded out according to the standard procedures, The Aqueous and methanolic extracts were subjected to preliminary phytochemical screening to identify the various phyto-constituents present in them i.e. alkaloids, terpenoids, glycosides, steroids, triterpenoids, flavonoids, carbohydrates, saponins and tannin **[11, 12]**.

A. Chemical test for alkaloids

Little quantity of dried extract with alcohol was shaken with dilute hydrochloric acid and filtered. The acidified filtrate was used to detect the presence of alkaloids by the following tests. **Mayer's test**

The acidified filtrate (2 ml) was treated with Mayer's reagent (1 ml), shaken well and observed for the presence of creamy precipitate.

Wagner's test

The acidified filtrate (2 ml) was treated with Wagner's reagent (1 ml) and observed for the presence of reddish-brown precipitate.

Hager's test

The acidified filtrate (2 ml) was treated with Hager's reagent (1 ml) and observed for the presence of yellow precipitate.

Dragendorff's test

The acidified filtrate (2 ml) was treated with Dragendorff's reagent (2 ml) and observed for the presence of orange-red precipitate.

B. Chemical tests for glycosides

Little quantity of dried extract was hydrolyzed with dilute hydrochloric acid on a water bath for a few hours, and the hydrolysate obtained was used to detect the presence of glycosides by following tests.

Legal test

The hydrolysate (2 ml) was dissolved in pyridine (2 ml). Freshly prepared sodium nitroprusside solution (2 ml) was added to it. Made the mixture alkaline with sodium hydroxide solution and observed for the formation of a pink color.

Baljet test

The hydrolysate (2 ml) was treated with sodium picrate solution (1 ml) and observed for the formation of a yellow to orange color.

Borntrager's test

A little quantity of the residue obtained from the evaporation of hydrolysate was mixed with water and shaken with an equal volume of chloroform. The chloroform layer was separated and equal quantity of dilute ammonia solution was added to it and shaken

well and observed for the formation of pink color in the ammoniacal layer.

Modified Borntrager's test

A little quantity of the residue obtained from the evaporation of hydrolysate was treated with ferric chloride and dilute hydrochloric acid. Then, it was extracted with chloroform. The chloroform layer was separated, and an equal quantity of dilute ammonia solution was added to it and shaken well and observed for the formation of pink color.

C. Chemical tests for Phenolic compounds and tannins

Ferric chloride test

A small quantity of the dried extract was mixed with water and treated with dilute ferric chloride solution (5%) and observed for the presence of a blue color.

Gelatin test

The dried extract dissolved in the water was filtered. To the filtrate, a 2% solution of gelatin containing 10% sodium chloride was added and observed for the presence of milky white precipitate.

Lead acetate test

The dried extract dissolved in the water was treated with a 10% lead acetate solution and observed for the presence of bulky white precipitate.

Decolorization test

The dried extract dissolved in water was treated with dilute potassium permanganate solution and observed for the decolorization of potassium permanganate.

D. Chemical tests for Flavanones and flavonoids

Aqueous sodium hydroxide test

Aqueous sodium hydroxide solution was added to the little quantity of dried extract and observed for the yellow coloration of the solution.

Ammonia test

The filter paper wetted with a small quantity of an alcoholic solution of the dried extract was exposed to ammonia vapor and observed for the formation of yellow color.

Shinoda test

The dried extract mixed with alcohol was treated with magnesium or zinc and dilute hydrochloric acid and observed for the formation of orange-red or violet color.

E. Chemical tests for carbohydrates

A small quantity of ethanolic extract was mixed with water or alcohol and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrates.

Molisch's test

The filtrate (2 ml) was treated with a few drops of Molisch's reagent and concentrated sulfuric acid (2 ml) was added through the side of the test tube without shaking and

observed for the presence of violet ring at the junction of two solutions.

Fehling's test

The filtrate (1 ml) was treated with 1 ml each of Fehling's solution A and B and boiled in a water bath and observed for the formation of a reddish precipitate.

Benedict's test

The filtrate (2 ml) was treated with Benedict's reagent (2 ml). Then, the mixture was heated in a boiling water bath and observed for the presence of reddish precipitate.

F. Chemical tests for proteins and amino acids

Millon's test

Little quantity of dried extract was treated with of Millon's reagent (2 ml) and observed for the formation of white precipitate, which on warming turn into a red colored solution.

Biuret test

Little quantity of dried extract was treated with a few drops of 2% copper sulfate solution. To this excess of potassium hydroxide solution was added and observed for the formation of violet colored solution.

Ninhydrin test

Little quantity of dried extract was treated with few drops of ninhydrin solution and heated on a water bath and observed for the presence of a violet color.

G. Chemical test for terpenoids

Salkowski test

Little quantity of dried extract was dissolved in chloroform. An equal volume of concentrated sulfuric acid was added to it and observed for the appearance of red color in the chloroform layer and greenish-yellow fluorescence in the acid layer.

H. Chemical tests for sterols

A little quantity of the alcoholic extract was refluxed with alcoholic potassium hydroxide solution until the saponification was observed. The mixture was diluted and extracted with solvent ether. The ethereal extract was evaporated, and the residue obtained was used in the tests for sterols.

Liebermann–Burchard test

The residue was taken with dry chloroform (1 ml), and then it was mixed with 2 ml of specially distilled acetic anhydride followed by a few drops of concentrated sulfuric acid through the sides of the test tube and observed for the formation of green color in the upper portion which changes to bluish violet.

Salkowski test

The residue was dissolved in chloroform, and an equal volume of concentrated sulfuric acid was added to it and observed for the red color in the lower layer.

I. Chemical tests for saponins

Foam (froth) test

A small quantity of dried extract was diluted with distilled water (20 ml) in a graduated cylinder. The suspension was shaken for 15 min and observed for the formation of froth.

Hemolysis test

A drop of blood was placed in a slide and mixed with a small quantity of dried extract and observed for hemolysis.

J. Chemical tests for gum and mucilage

Absolute alcohol (25 ml) was added with an aqueous extract (10 ml) with constant stirring. Filtered and the precipitate formed was dried in air and examined for swelling properties.

K. Chemical test for volatile oil

Powdered material (50 g) was subjected to hydro-distillation in volatile oil estimation apparatus (Clevenger apparatus). Collect the distillate and observed for the presence of volatile oil layer.

***In vitro* antioxidant assay**

DPPH radical scavenging activity

These are most identification part to identify which types of antioxidant are present in plant . The antioxidant bustle of all mine was exact in requisites of hydrogen donate or free radical scavenging movement, via the sure radical DPPH. DPPH's (Di – phenyl – Picryl hydrazine) scavenging activity we referred the method of Gulcin J. *et al* 2006 and R. Jain *et al* 2006. According to these methodologies DPPH solution was prepared 40 microgram/ml solution **[13]**. Now prepared different dilution of extract as 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 mg/ml. in these solution were added 2ml of DPPH solution. These solutions were leave for incubation at room temperature at 10 minutes. After this spectrophotometer was leave for warming. Absorbance was measured at 517nm. Calculate percentage inhibition and $IC_{50.}$

Percentage inhibition = Ac – At*100/Ac

Ac = Absorbance of control

At = Absorbance of test

Hydrogen Peroxide assay

Hydrogen peroxide assay was performed according to Jayaprakash G.K. *et al* 2013 and Ruch R.J. *et al* 1989. According to both author prepared different dilution of extract. This dilution was 5 to 25 µg/ml. 2ml of test sample and 1ml of 20mMHydrogen peroxide solution in phosphate buffer saline 7.4. Spectrophotometer was calibrated by using same solvent. Wavelength was set 230nm of spectrophotometer. Measured the absorbance of these samples and calculate the percentage inhibition **[14]**.

Percentage inhibition = Ac – At*100/Ac

Ac = Absorbance of control

At = Absorbance of test

Total Phenolic content

Determination of total phenol content was carried out by Folin- Ciocalteu reagent method. 0.5 ml of extract (1:10 µg/ml) or Gallic acid was mixed with 5 ml Folin-Ciocalteu reagent (1:10 distilled water) and 4 ml of aqueous Na_2CO_3 (1 M). The mixtures were left at room temperature for 15 min and the total phenol content was determined by colorimetric method at 765 nm. Total phenol content was calculated from calibration curve and expressed as Gallic acid equivalent (mg GAE/g) **[15]**.

Total Flavonoid content

The total flavonoid content was estimated using $AlCl_3$ method. 0.5 ml of each extract (1:10 µg/ml) in methanol were mixed with 1.5 ml of methanol, 0.1 ml of $AlCl_3$ (10%), 0.1 ml of KCH_3COO (1 M) and 2.8 ml of distilled water and left at room temperature for 30 min. Absorbance of the mixtures was measured at 415 nm. Total flavonoid content was calculated from calibration curve and expressed as Quercetin equivalent (mg QE/g extract) **[16]**.

Administration, symptoms recorded during study

Acute oral toxicity studies were perform as per OECD guideline. Albino wistar rats (n = 6/each dose) were used. The animals were keep fasted over 12 hours. In these duration animals were weighed and test extract was administrated orally at a dose of 5, 50, 100, 500, 1000, 2000, 3000, and 4000 mg/kg. After the administration of extract, food for the animals were in remission for 2 hours. The mortality and clinical signs noted which incorporated changes in skin, fur, eyes and mucous membranes were noted for the first 5 hours then for 72 hours and next 7 days of test drug administration. For complete 7 days, the gross behaviors like body positions, locomotion, rearing, tremors and gait were observed and also the effect of plant extract on grip strength, pain response and righting reflex were noted.

RESULT AND DISCUSSION

The Phytochemical analysis *wheat daliya* were revealed the presence of various chemical constituents such as alkaloids, saponins, glycosides, tannins, flavonoids, carbohydrate etc **(Table 1, 2)**.

Phytoconstituents detection of both aq extract concluded that extract major Phytoconstituents which is helpful for further studies, it possess carbohydrate, protein, amino acids, Phenolic compounds, flavonoids, Saponins etc.

A number of research have resulted that free molecules in bodies are responsible for lot of rare disease originate. These are as

related to immunity, nervous system dysfunction, cardiovascular disease and may be carcinogenic etc. DPPH is a constant free radical at opportunity at room temperature. It has posses both properties in which accept an electron or hydrogen radical near suit a sure diamagnetic molecule. DPPH-H. IC_{50} of DPPH is major role play in measuring antioxidant activity. Aq. extract have IC_{50} value was 24.60mg/ml Ascorbic while both whole grain and brown rice 14.28 mg/ml, 24,28 mg/ml. so that these compound have posse's good antioxidant activity **(Table 3-5)**.

Hydrogen peroxide scavenging activity of *Aq* extract were determined. Resulted showed that it is a concentration dependent activity against hydrogen peroxide with IC_{50} values of 62.34 mg/ml, 52.56 mg/ml. Ascorbic acid used as standard its inhibition value was 22.73 µg/ml (**Table 6**).

Total phenolic content assay presentation is main aim is plant has high amount of Phytochemicals are present. Phenolic compound have posses high number of free radicals. Many of reasons in which broad therapeutic activity. Total phenolic content in Aqueous extract have high level of phenolic content is present. Aq extract posses 139mg/gm, Brown rice 110 mg/mg of total phenolic content of 100 mg/mg.

Phenolic compound and flavonoids are mainly present in natural sources. After reading many of research article resulted that in natural plant obtained sources. Phenolic compound is higher number than flavonoids because it is part of phenolic component. In these series it may be flavonoid, isoflavonoids, terpenes etc. Aq. Extract have posses good flavonoids component. It has in whole grain aq extract 107mg/gm while brown rice 68 mg/ml. These result show in minute concentration of sample that was 100mg/mg.

Acute toxicity will power is a way for assess acute oral toxicity that involve the appreciation of a dose level that cause mortality. The dose restrictions were chosen on the base of oral acute toxicity study in rats according to OECD strategy. The acute toxicity examination was performed in albino wistor rats by administring different doses of aq extract i.e. 5,50,100, 1000, 2000 , 3000 and 4000 mg/kg body weight. Parameters ruleout as per mentioned in **Table 7, 8**. All groups of animals showed neither any toxic effect, nor any lethal effect. Therefore, LD50 of extract may be considered to be greater than 4000 mg/kg.

Table 1: Phytochemical Analysis of Whole Grain

Phyto constituents	Chemical tests	Aqueous
Alkaloids	Dragendroffs	+
	Hager's test	+
	Mayer' test	+
	Wagner's test	+
Carbohydrate	Molish test	-
	Fehling's test	+
	Benedict's test	+
	Barfoed's test	-
Proteins	Biuret's test	+
	Milion's test	-
	Precipitation test	+
Amino tests	Ninhydrin test	+
	Xanthorproteic test	+
Steroids	Salkowski's test	-
Flavonoids	Shinoda test	+
Glycoside	Brontrager's test	+
	Legal's	+
Tannins and Phenolic	Zinc HCl test	-
	With 5% ferric chloride	-
	With KMnO4	-
	With lead acetate	-

Table 2: Phytochemical Analysis of Brown Rice

Phyto constituents	Chemical tests	Aqueous
Alkaloids	Dragendroffs	+
	Hager's test	+
	Mayer' test	+
	Wagner's test	+
Carbohydrate	Molish test	-
	Fehling's test	+
	Benedict's test	+
	Barfoed's test	+
Proteins	Biuret's test	+
	Milion's test	-
	Precipitation test	+
Amino tests	Ninhydrin test	+
	Xanthorproteic test	+
Steroids	Salkowski's test	+
Flavonoids	Shinoda test	+
Glycoside	Brontrager's test	+
	Legal's	
Tannins and Phenolic	Zinc HCl test	+
	With 5% ferric chloride	+
	With KMnO4	+
	With lead acetate	+

Table 3: Curve of Ascorbic Acid

S.No.	Conc. (3g/ml)	% inhibition	R^2	IC_{50}
1	0.2	12.12		
2	0.4	21.4		
3	0.6	33.0	0.9714	24.60
4	0.8	57.73		
5	1.0	73.70		

Table 4: Aq Extract Whole Grain

S.No.	Concentration (µg/ml)	% inhibition	R^2	IC_{50}
1	0.02	25.23		
2	0.04	32.78		
3	0.06	50.23	0.985	14.28
4	0.08	63.2		
5	0.1	75.23		
6	0.2	86.76		

Table 5: Aq Extract Whole Grain

S. No.	Concentration (µg/ml)	% inhibition	R^2	IC_{50}
1	0.02	18.22		
2	0.04	32.18		
3	0.06	52.20	0.965	24.17
4	0.08	68.2		
5	0.1	82.23		
6	0.2	96.62		

Table 6: Hydrogen peroxide scavenging activity of Ascorbic acid

S. No.	Concentration (µg//ml)	% inhibition	R^2	IC50
1	2.5	2.35		
2	5.0	4.26		
3	7.5	9.59		
4	10	22.67	0.944	22.95 microgm/ml
5	12.5	25.81		
6	15	29.21		

Table 7: Effect of extract whole grain on acute oral toxicity test in Albino Wistar rats

S. No.	Response	Before treatment	After Treatment 5 mg/kg	After Treatment 10 mg/kg	After Treatment 50 mg/kg	After Treatment 100mg/kg	After Treatment 1000mg/kg	After Treatment 2000mg/kg	After Treatment 3000 mg/kg	After Treatment 4000mg/kg
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	Grooming	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
3.	Restlessness	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
4.	Touch response	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
5.	Pain Response	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
6.	Tremors	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
7.	Convulsion	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
8.	Griping	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9.	Pinna Reflex	Present	Present	Present	Present	Present	Present	Present	Present	Present
10.	Pupils	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11.	Urination	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
12.	Skin colour	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
13.	Food intake	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
14.	Water Intake	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
15.	Mortality	NA	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table 8: Effect of aq extract Brown rice on acute oral toxicity test in Albino Wistar rats

S. No.	Response	Before treatment	After Treatment 5 mg/kg	After Treatment 10 mg/kg	After Treatment 50 mg/kg	After Treatment 100mg/kg	After Treatment 1000mg/kg	After Treatment 2000mg/kg	After Treatment 3000 mg/kg	After Treatment 4000mg/kg
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	Grooming	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
3.	Restlessness	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
4.	Touch response	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
5.	Pain Response	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
6.	Tremors	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
7.	Convulsion	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
8.	Griping	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9.	Pinna Reflex	Present	Present	Present	Present	Present	Present	Present	Present	Present
10.	Pupils	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11.	Urination	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
12.	Skin colour	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
13.	Food intake	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
14.	Water Intake	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
15.	Mortality	NA	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

CONCLUSION

Both aq. Possess good number of pytoconstituent, Free radical and nonoff toxicity.

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