WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 8, 1092-1099. Research Article ISSN 2277 – 7105

PHYTOCONSTITUENT STUDY OF BROWN RICE

M. Krishnaveni* and R. Dhanalakshmi

*Assistant Professor, Department of Biochemistry, Periyar University, Salem-636 011.

M. Phil Student. Department of Biochemistry, Periyar University, Salem-636 011.

Article Received on
09 August 2014,

Revised on 06 August 2014,
Accepted on 29 Sept 2014

***Correspondence for Author**
Dr. M. Krishnaveni
Assistant Professor, Department of Biochemistry, Periyar University, Salem.

ABSTRACT

Brown rice is whole grain rice, reduces insulin and glycemic indices, and also offers other health benefits. Considering the benefits offered by brown rice as reported in the literature, it was decided to conduct a preliminary research on the qualitative, quantitative aspects of it. Hence, brown rice was studied for its phytochemicals, behavior with different chemicals, fluorescence nature, percent yield, nutrient content. Brown rice contain phytochemicals such as carbohydrate, alkaloid, glycoside, tannin, phenolic compound, flavonoid, steroid, sterol, saponin, protein, amino acid, fixed oil, starch, anthroquinone. The percent yield was found to be 10.50 and was fluorescing yellow in color. Total carbohydrate, protein, amino acid content calculated was 179.33±1.15mg/g, 1.46±0.23mg/g, 3.32±0.09mg/g. From the results obtained, it is concluded that is rich in phytochemicals with therapeutic ability.

KEY WORDS: Aqueous extract, Brown rice, Fluorescence, Nutrients, Phyto-constituent.

INTRODUCTION

Rice is one of the most important crops in the world. It is consumed as a staple food by more than half of the world's population and approximately 95% of its production is in Asia. Rice is considered a principal dietary component, particularly in the Asian countries, where per person daily rice consumption may be up to 0.5kg dry weight. [1, 2] Probiotic foods available in the market are mostly prepared from milk based, but nowadays, other substrate apart from milk is also used. One such substitute is rice, as it is capable of supporting the growth of probiotic bacteria through its broth, offering protective effect of bile resistance. [3] Brown rice is superior to other polished rice as it has a high dietary fiber- a mild laxative, prevents gastro-intestinal disorders, healthy food for diabetic patient. [4] The total sugar released in

vitro was 23.7% lower in brown rice than in milled rice. The brown rice contains magnesium a mineral, which acts as a cofactor for more than 300 enzymes. About 5% of the deaths that occur globally each year are mainly due to disease, almost 80% of which occur in low, middle income countries.[5,6] Hence, the present study was planned to investigate the phytochemicals as well as other basic nutrients present in the brown rice.

MATERIALS AND METHODS

Sample Collection

The Brown rice was purchased from supermarket at Chennai, Tamil Nadu, India during Feb, 2014. The purchased rice were cleaned thoroughly and ground to powder using blender for further use.

Aqueous Extract Preparation

Aqueous extract was prepared by dissolving 15g of powdered Brown rice in 200ml of distilled water. The mixture was heated on a hot plate with continuous stirring at 30-40°C for 20minutes. Then the water extract was filtered through filter paper. The filtrate was kept in a beaker and allowed to dry by heating in a boiling water bath. The gummy residue obtained was used for the analysis of percentage yield, and the remaining marc left was extracted with water and used for qualitative analysis.

Phytochemical Analysis

The extract was tested for the presence of bioactive compounds by adopting standard procedures. [7, 8] fluorescence analysis,[9] behavior of drugs powder with different chemical reagents. [10]

Test for Carbohydrate

Molisch's test: To the extract added few drops of alcoholic alpha naphthol solution followed by few drops of concentrated sulphuric acid along the sides of the test tube. Purple or violet colored ring formed at the junction showed positive result.

Fehling's Test: To the extract added equal amount of Fehling's A and B solution, heat the tubes in a boiling water bath. Brick red precipitation of cuprous oxide formation confirmed the presence of reducing sugar.

Benedict's Test: To the extract added Benedict's reagent and the contents in the tubes were heated in a boiling water bath. Formation of red precipitate indicated positive result.

Test for Alkaloid

Wagner's Test: To the extract added few drops of iodine solution in potassium iodide. Reddish brown precipitate showed positive result.

Hager's Test: To the extract added few drops of saturated solution of picric acid. Yellow color precipitation showed positive result.

Test for Steroid and Sterol

Libermann-Burchard Test: To the extract added 2ml chloroform, 10 drops of acetic anhydride, 2 drops of concentrated sulphuric acid. Positive result was confirmed by the formation of bluish red to cherry red color in chloroform layer.

Salwoski Test: To the extract, few drops of chloroform were added followed by concentrated sulphuric acid. Presence of bluish red to cherry red color denoted positive result.

Test for Glycoside

Legal Test: To the extract added pyridine and sodium nitroprusside. Positive result showed pink red color formation.

Baljet Test: To the extract added picric acid. Appearance of orange color showed positive result.

Test for Saponin

Foaming test: Foams produces when the extract was mixed with water.

Test for Flavonoid

Shinoda Test: To the extract added magnesium turnings, 1-2 drops of concentrated hydrochloric acid. Appearance of red color indicated positive result.

Zinc Hydrochloride Test: To the extract added zinc dust, 1-2 drops of concentrated hydrochloric acid. Appearance of red color indicated positive result.

Test for Tannin and Phenolic Compound

Ferric Chloride Test: To the extract added ferric chloride. Appearance of greenish black color showed positive result.

Potassium Dichromate Test: To the extract added potassium dichromate solution. Positive result was confirmed by the formation of brown precipitate.

Gelatin Test: To the extract added 1% gelatin solution containing 10% sodium chloride. Formation of white precipitate showed positive result.

Test for Protein and Amino Acid

Biuret Test: To the extract added 4% sodium hydroxide followed by few drops of 15% copper sulphate. Appearance of purple color showed positive result.

Ninhydrin Test: Bluish violet color was formed when a solution of ninhydrin was added to the extract and heated.

Heat Test: The extract was heated by allowing it to boil in a water bath. Presence of coagulation showed positive result.

Test for Fixed Oil

Copper Sulphate Test: Blue color was observed when the extract was mixed with 1ml of 1% copper sulphate and 10% sodium hydroxide.

Quantitative Analysis of Phytonutrients

Total carbohydrates, [11] proteins,[12] amino acids[13] were performed according to the standard prescribed methods.

Estimation of Carbohydrate

The total carbohydrate was estimated by anthrone method**.** 1mg of brown rice powder was hydrolyzed to simple sugars by keeping it in a boiling water bath for three hours with 5ml of 2.5N HCl and cooled to room temperature. After neutralizing, the contents were centrifuged and 0.1 ml of supernatant was used for the analysis. To the sample add 4ml of anthrone reagent and the contents were heated in a boiling water bath for 8 minutes. The tubes were cooled and read at 630nm using spectrophotometer Schimadzu Model - UV 1800. The standards were developed with glucose. Standard graph plotted was used to find the concentration of glucose present in the unknown/ sample.

Estimation of Protein

The total protein was estimated by Lowry's method. To 0.1ml of extract added 2ml of alkaline copper reagent, mixed well and incubated for 10minutes. After the incubation period 0.2ml of Folin ciocalteau reagent (diluted in the ratio of 1: 2) was added and allowed for 30minutes incubation, then read at 660nm using spectrophotometer Schimadzu - Model UV 1800. The standards were developed with Bovine serum albumin. Standard graph plotted was used to find concentration of protein present in the unknown/ sample.

Estimation of Aminoacids

The amino acid was estimated by Ninhydrin method. To 0.1ml of sample added 1ml of ninhydrin solution dissolved in ethanol. The test tube was covered with a piece of paraffin film to avoid the loss due to evaporation. With gentle stirring, react at 80-100°C for 4-7 minutes. The test tubes cooled and the color developed was read at 570nm. Tyrosine was used for developing standards.

Statistical Tool

Each experiment was carried out in triplicate and the results are given as Mean ± Standard deviation. The Mean and Standard deviation (S) was calculated by using the following:

Formula: Mean = Sum of x values / n (Number of values), $s = \frac{\sqrt{\sum(X-M)^2}}{n-1}$

RESULTS AND DISCUSSION

The yield calculated was tabulated in Table.1.

Table.1 Percentage yield of Brown rice aqueous extract

S.No	Name of the powder	Weight taken for extraction	Initial weight of the beaker (gm)	Final weight of the beaker (gm)	Weight of the extract Powder (gm)	Recovery (%)
1.	Brown rice	15g in 200ml water	167.5915	169.1680	1.576	10.50

The percentage recovery of the aqueous extract obtained was calculated and expressed in Table 1. The percent recovery was found to be 10.50.

Analysis of brown rice powder for its behavior

Table. 2 Behavior of Brown Rice Powder with Different Chemicals

S.No	Tests	Observation	Inference
1.	Powder + Picric acid	Yellow color	Presence of alkaloid
2.	Powder + Conc. H_2SO_4	Reddish brown color	Presence of steroids

3.	Powder + Aqueous $FeCl_3$	Yellow color	Presence of Flavonoids
4.	Powder + Iodine solution	Blue colour	Presence of starch
6.	Powder + Aqueous 5% KOH	No yellow color	Presence of anthroquinone
7.	Powder + NaOH	No yellow color	Absence of flavonoid
8.	Powder + Aqueous $AgNO_3$	White precipitate	Presence of protein

The result of brown rice powder behavior is shown in Table. 2. The behavior of brown rice powder with different chemicals showed positive result for alkaloid, steroid, anthroquinone, protein, starch.

Fluorescence Analysis

Table.3 Fluorescence Analysis of Brown Rice Aqueous Extract

S.No	Name of the extract	Day light	UV light
1.	Aqueous	Pale pink	Yellow fluorescence

The fluorescence analysis results are depicted in Table. 3. The brown rice powder extracted with water was pale pink in color when observed under ordinary day light. The same when viewed under UV light showed yellow fluorescence.

Phytochemical Analysis

The results of phytochemical analysis are shown in Table.4.

Table.4 Phytochemicals in Brown Rice Aqueous Extract

S. No	Name of the test	Results
1.	**Test for carbohydrate** a)Molisch's test b)Fehling's test c)Benedict's test	 +++ +++ +++
2.	**Test for alkaloid** a)Wagners test b)Hagers test	 +++ ++
3.	**Test for steroid and sterol** a)Libermann - Burchard test b)Salwoski test	 + ++
4.	**Test for Glycoside** a)Legal test b)Baljet test	 +++ ++
5.	**Test for saponin** Foam test	 +
6.	**Test for flavonoid** a)Shinoda test b)Zinc hydrochloride test	 + -

7.	**Test for tannin and phenolic compound** a)Ferric chloride test b)Potassium dichromate test c)Gelatin test	 ++ ++ +
8.	**Test for protein and amino acid** a)Biuret test b)Ninhydrin test	 ++ ++
9.	**Test for fixed oil** a)Copper sulphate test	 ++

+Slight changes, ++ Moderate, +++ Stronger reactions

Positive result was observed for carbohydrate, alkaloid, steroid, glycoside, saponin, flavonoid, tannin, phenolic compound, protein, amino acids. (Table.4)

Phytonutrient Analysis

The phytonutrients estimated were tabulated in Table. 5.

Table.5 Phytonutrients in aqueous extract of Brown rice

S.No	Phytonutrients	Nutrient content (mg/g)
1.	Total carbohydrate	179.33±1.15
2.	Total protein	001.46±0.23
3.	Amino acids	003.32±0.09

Values are Mean ± SD for three experiments

The total carbohydrate content observed was 179.33±1.15mg/g. Likewise, the total protein, amino acid content was found to be 1.46±0.23mg/g, 3.32±0.09mg/g. Here, carbohydrate content was higher when compared to total protein and amino acid content. The brown rice has been reported for its antioxidant activities by krishnaveni et.al. [14]

CONCLUSION

Brown rice is a nutritionally important food source and the demand for it increases today. The health benefits of brown rice is not known to everyone as it is an expensive than common white rice and at the same time brown rice was not consumed by everyone commonly. Considering, the health significance, it was assessed, the results obtained show, that brown rice contain phytochemicals such as carbohydrate, alkaloid, glycoside, phenolic compounds, tannin, saponin, flavonoid, protein, amino acid qualitatively and when assessed quantitatively it was found to be rich in carbohydrate but the content of amino acid, protein was low compared to carbohydrate. From this we can conclude, that brown rice is endowed with phytochemicals which might be helpful in acting as a potential drug of future.

ACKNOWLEDGEMENT

The author wishes her thanks to Honorable Vice-chancellor Dr. C. Swaminathan Avl and Registrar Dr. K. Angamuthu Avl, Periyar University, Salem for their administrative support and excellent infrastructure facilities provided and also thank Dr. V. Raj, Professor and Head, Department of Chemistry, Periyar University, Salem for his help.

REFERENCES

1. Zavala YJ, Duxbury J M. Arsenic in rice: I. Estimating normal levels of total arsenic in rice grain. Environ. Sci. Technol., 2008; 42: 3856−3860.
2. FAO. Rice Information; FAO: Rome, Italy, 2002; 3.
3. Charalampopoulos D, Wang R, Pandiella SS, Webb C. Application of cereals and cereal components in functional foods: A review. International Journal of Food Microbiology, 2002; 79: 131–141.
4. Garrow JS, James WPT, Ralph A. Human Nutrition and Dietetics, 10th edition, Churchill Livingstone, Harcourt Publishers, London, 2000.
5. International Diabetes Federation. Diabetes Atlas: The Global Burden, 2011.
6. World Health Organization. Diabetes Fact sheet, 2008.
7. Harborne JB. Phytochemical methods, 2nd edition, Chapman and Hall, New York, 1984.
8. 8.Kokate CK, Purohit AP, Gokhale SB. Pharmacognosyy, 3rd edition, Nirali Prakashan, Pune, 1995.
9. Kokoshi CL, Kokoshi RJ, Sharma FJ. Fluorescence of powdered vegetable drugs under UV Radiation. J Am Pharm Assoc., 1958; 47: 715-717.
10. Chase CR, Pratt RJ. Fluorescence of powder drugs with particular reference to development of a system of identification. J Am Pharm Asso., 1949; 38: 324-331.
11. Hedge JE, Hofrelter BT. In: Carbohydrate chemistry, 17 Eds. Whistler RL, Be Miller JN, Academic press, New York, 1962.
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol Reagent. J Biol Chem., 1951; 193: 265-275.
13. Yemm EW, Cocking EC, Ricketts RE. The determination of amino acids with ninhydrin, Analyst, 1955; 80: 209-214.
14. 14.Krishnaveni M, Magesh P, Jasbin shyni G, Dhanalakshmi R, Ponraj K, Lavanya K, Kalimuthu R. International journal of pharmaceutical sciences review and research, 2014;134-137.