IJPSR (2017), Volume 8, Issue 7 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 December, 2016; received in revised form, 07 February, 2017; accepted, 17 February, 2017; published 01 July, 2017

AN *IN VITRO* COMPARATIVE STUDY ON ANTIOXIDANT, ANTIBACTERIAL AND NUTRACEUTICAL PROPERTIES OF THREE DIFFERENT COLOURED SCENTED RICE VARIETIES OF NORTH- EASTERN REGION

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Keywords:

Antioxidant, Black rice, *Staphylococcus* strain, Nutraceutical content, Methanol extract

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ABSTRACT: The present study was conducted to evaluate the antioxidant, antibacterial and nutraceutical properties of three coloured scented rice varieties grown in North Eastern region. The study for the analysis of Nutraceutical content involved more than five contents to find a significant correlation of the all the rice varieties. Antioxidant activity was measured using hydrogen peroxide and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays. The antioxidant activities of the studied samples were expressed as percentage (%) of DPPH and H_2O_2 radicals inhibition and IC_{50} value. Among different extracts of crude rice, methanolic extract of the black rice showed highest antioxidant activity compared to the aqueous extract of the other two varieties each for DPPH (89.29±0.139) as well H_2O_2 (80.97±0.091) with an IC_{50} value of 40 µg/ml and 42.6µg/ml respectively. Also the result of MIC against the tested *staphylococcus* strain was notably found to be effective with a significant value of 119 µg/ml for black rice taken DMSO as the positive control.

INTRODUCTION: North-East India particularly Assam, is rich in production of coloured and scented rice, a hulled grain with a distinctive red or purple colour in addition to light grey on its bran. Of all the coloured rice, especially black rice has long been consumed and is considered as a healthy food in Korea [11]. Although, the coloured rice are hard in its cooking texture, they possess a beneficial effects of coloured pigment, the naturally occurring coloured substances like anthocyanin that belongs to flavonoid family which is reported to combat against the damaging effect of toxic free radicals [32] and has great pharmacological property [27].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(7).2968-74

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (7).2968-74

Equally interesting is this food for the elimination of a series of other problems including obesity, edema, hypertension, diseases of kidneys and diabetes by releasing glucose in a fairly moderate way and constant way, which allow to stabilize the sugar level in the blood (www.medicinal properties of rice.com). The presence of oryzanol in the whole rice also ensures its affectivity in reducing cholesterol level (LDL) in blood.

In external use, it serves as a good anti-inflammatory as well as astringent in its crushed and dried form. With its potent bio-active compounds such as phenolic compounds, tannins, lignin, oryzanol, tocotrienols, tocopherols, phenyl propanoids, and flavonoids [29], the coloured rice(s) are responsible for counteracting wide range of illness. Rice with its active components is often determined as a rich source of nutraceuticals which commonly means "Nutrition" and "Pharmaceutical" [20] and coloured rice profoundly rich in iron (Fe), zinc (Zn), and minerals, Vitamin

A, Vitamin B, and Vitamin E which are beneficial for special prevention of heart disease and overall considered to be more nutritious. The reported nutraceutical content in Chakhao, the Manipuri aromatic black glutinous rice of North east India has been reported with great value for human health benefit and the huge demand for market also. The paper also reports the antimicrobial activity of rice origin which has been claimed with enormous therapeutic potential and effective in alleviating many of the side effects that are often associated with synthetic antimicrobials.

These preparations hailed the effectual remedy of phenolic compound and flavonoids as active constituents for the treatment of human diseases and in anti-infective research Indian Ayurveda. Rice powder and pollutice were the externally applied talc before the production of modern talcum powder and various cream in case of measles, smallpox, prickly heat, and other skin infection or skin inflammatory infection such as scalds, and burns [16]. The reported good and powerful antibacterial activity of colored rice crude extract is against causing skin and soft tissue infection bacteria such as *Staphylococcus aureus* [33]. Rice bran oil which have great medicinal and pharmaceutical properties are widely used have been also indulged in the antibacterial activity against human pathogens [23].

MATERIALS AND METHODS: DPPH (2, 2-diphenyl-1-picryl-hydrazyl) and hydrogen peroxide (30%) were purchased from Sigma-Aldrich (USA). The bacterial strains were identified and collected from Downtown hospital (Assam, India). All other chemicals and solvents were of HPLC grade. De-ionized water was used throughout all the experiments. The rice samples were collected from different areas of North-East India in the month of November and December, 2015. The species were authenticated with the standard herbarium specimen in the Department of Botany, Gauhati University. The collected samples were air dried and powdered. The extraction process was performed by Soxhlet method with methanol as a solvent. 20gm of power was weighed and placed in the thimble holder of the apparatus for 5-6 hours. The aqueous extract was prepared by dissolving the dried grinded rice sample (30g) in 1000ml of distilled water. The mixture was boiled for 15 minutes at 100 ºC in a hot water bath, cooled and filtered using Whatman's filter paper. The filtrate thus obtained was used for the analysis.

Nutraceutical content Analysis:

Estimation of moisture and dry matter content: Approximately 25 gram of grinded rice sample(s) were weighed (W_1) on an electronic balance and was kept in the electronic oven for 12 hours at 105 ºC [12]. Then after the samples were cooled in desiccators and weighed (W_2). The loss of percentage in weight was expressed as a percentage of total moisture contents.

The percentage of moisture content was calculated by using the formula:

$$\% \text{ of moisture content} = W_1 - W_2 / W_1 \text{ X } 100$$

Estimation of ash content: The determination of the ash content from different grinded rice sample(s) was done following the method of AACC [12] with slight modifications. The clean and dried china dishes containing 2 grams of the grinded samples were pre weighed (W_1). All the dishes were placed in an electronic oven at 150 ºC for 33 hours. After 33 hours of heating and cooling, the removed china dishes were weighed again as (W_3). Weight of the empty dish was taken as (W_2).

The percentage loss in weight were expressed as the ash content and calculated by the formula:

$$\% \text{ of ash content} = W_2\text{-}W_3 / W_1 \text{ X } 100$$

Estimation of crude fibre: Estimation of crude fibre of the grinded rice sample(s) was done by following acid-base digestion method of Aberoumand [13] and AACC [12] with few modifications. 2gm of the grinded sample(s) were treated with 200ml of 1.25% Sulphuric acid for 30 min using bumping chips. After treating with acid, the solutions were filtered using muslin cloth and the residues were washed for three times using 50 ml aliquots of boiling water followed by treatment with 200 ml of 1.25% NaOH and boiling for 30 min. The obtained solution were again filtered and washed by aliquots solution. The final washes were given using 25ml of alcohol. The obtained residues were put in pre-weighed (W_1) china dishes and placed in electronic oven at 130 ºC for 2 hour.

After two hours of heating and cooling, china dishes were weighed as W_2.

For ignition, the china dishes were treated at 300 ºC for 1 hour and reweighed as W_3 after proper cooling. The percentage of loss in weight on ignition, which expresses the crude fibre content, was calculated as:

$$\% \text{ CFb} = W_2\text{-}W_3 / W_1 \text{ X } 100$$

Estimation of fats: Crude fats extraction from grinded rice sample(s) was determined using soxhlet method recognized by AACC [12]. Grinded sample (20 grams) in filter paper was placed in the thimble holder of the apparatus and extracted with petroleum ether for 5-6 hours. Then after, weight of the empty round bottom flask was taken as W_1 and weight of flask with the extract was taken as W_2. The percentage of extracted fats was calculated as:

Weight of empty round bottle: W_1
Weight of oil (after extraction) round bottle: W_2
Weight of oil: $(W_1\text{-}W_2) = W_3$

$$\text{Oil in ground sample\%} = W_3 / 20 \text{ x } 100$$

Estimation of protein: The total protein content in the sample(s) was estimated by following the method of Lowry. To 0.5 ml of the extracts, 5ml of copper reagent, mixture of solution A (2% Na_2CO_3 in 0.1N NaOH) and solution B (0.5% CuSO4 solution in 1% Sodium Potassium Tartarate) was added. The contents of the tubes were mixed by shaking the tubes and were allowed to stand for 10 min at room temperature. 0.5ml of diluted Folin-Ciocalteau reagent was added to all the contents and mixed thoroughly. Absorbance was read at 660nm against an appropriate blank. Bovine serum albumin was taken as a standard.

Estimation of Carbohydrate: The carbohydrate estimation of the rice samples were done following Anthrone's method. To 1ml of the extracts, 4ml of Anthrone reagent (0.2% Anthrone reagent in 95% chilled H_2SO_4) was added carefully. The tubes were boiled in water bath for 10 min and were cooled at room temperature and the absorbance was read at 630 nm against an appropriate blank.

Nutritive value/energy value: The nutritive/ energy value of the samples were calculated by following the method of Effiong and Udo [24] and AOAC1 [8]. Nutritive value in kilocalorie per gram (kcal/g) was determined by multiplying the total value of percentage of fats, carbohydrates, and proteins by the recommended factors of 9, 4, and 4 respectively. The values obtained after multiplying were then added to get the sum which was then multiplied by 4.2 to change the unit to kilojoules.

Antioxidant Activities:

DPPH scavenging activity: The DPPH scavenging activity of methanol and aqueous extract of the sample(s) were assayed with slight modifications according to the method of Kaur *et al.,* [1]. The test sample of different concentration (10 µg/ml-160 µg/ml) was prepared and the final volume was made 3ml with DPPH and distilled water. The absorbance was determined at 517 nm after incubation of 30min at room temperature against an appropriate blank. Ascorbic acid was taken as standard.

The DPPH radical scavenging assay of the different rice sample was calculated by the formula:

$$\% \text{ inhibition} = A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}} \text{ x } 100$$

IC_{50} is defined as the concentration of substrate that causes 50% loss of the DPPH activity (colour) [13].

Hydrogen peroxide scavenging activity: The Hydrogen peroxide Scavenging Activity of methanol and aqueous extract of the sample(s) were assayed according to the method of Ruchet *et al.,* [34] considering slight modifications. The sample extracts of concentration (10 µg/ml-160 µg/ml) were made upto a total volume of 3ml by mixing with 600 µl of H_2O_2 solution and distilled water. After an incubation of 10 minutes at room temperature, absorbance was taken at 230 nm using phosphate buffer as a blank.

The percentage of hydrogen peroxide scavenging is calculated as follows:

$$\% \text{ scavenging} = A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}} \text{ x } 100$$

Antibacterial activity test:

Minimum Inhibitory Concentration: Minimum was added. A positive control with 1% inhibitory concentrations of all the extracts were determined using Clinical and Laboratory Standard Institute

broth micro dilution method (CLSI) with suitable modifications. Two fold serial dilutions were proposed for the extracts to obtain the concentration of 10mg/ml in five different test tubes. To these, 5ml of overnight culture broth of *Staphylococcus aureus* species DMSO and a negative control without an added inoculum were taken.

The test tubes were incubated at 37 ºC for 18 hours. After the incubation period was over, the MIC values were determined considering the lowest concentration of rice extract that causes complete inhibition of the bacterial growth.

Statistical analysis: All the assays were performed in triplicates. The data obtained were expressed as mean ± Standard deviation and analysis were done by calculating applied Student's t- test and one – way ANOVE at 95% confident level. The P-values ≤ 0.05 were considered statistically significant. The results were presented as the mean ± S.D.

RESULTS AND DISCUSSION:

Nutraceutical Content Analysis: In the present work, the nutraceutical content of three rice varieties of North Eastern region was revealed. The study involved more than five contents to find a significant correlation of the all the rice varieties. Results are in consonance with the findings of Handique *et al.,* [28] who worked with 14 indigenous scented rice for their nutritive values. However, the work done by Borthakur *et al.* ,[33] showed the crude fibre content for Joha rice varied from 1.29% to 2.32% which is considerably lower than our finding shown in **Table 1** *i.e*. 14.5%. Nutritive value or Energy value were found to be impressive in Black rice with a value of 300 Kcal/gm. The protein content was found to be higher in Black rice compared to a value of 5.2% in both brown and white rice as reported by Souci and his co-workers [35]. This is in contrast to the carbohydrate content which was observed significantly higher in the Joha rice variety.

TABLE 1: NUTRACEUTICAL CONTENTS OF THE RICE VARIETIES

Samples	Moisture content%	Fibre content%	Ash content%	Fat content%	Protein Content (µg/ml)	Carbohydrate Content (µg/ml)	Nutritive/Energy value (Kcal/gm)
Black	11.84±0.80	35±0.81	7.5±0.73	1.4±0.24	31±1.63	41±0.72	300.6±0.24
Joha	13.28±1.62	14.5±0.32	10±0.77	0.5±0.03	13±1.26	45±0.84	236.5±1.14
Bora	14.6±0.40	3.5±0.18	4±0.23	0.7±0.13	11±0.89	35±0.57	190.3±1.11

Antioxidant Activity Analysis: The antioxidant activity for each of the crude methanol and aqueous extract was determined with DPPH free radical scavenging and H_2O_2 radical scavenging. The data shown in **Table 2** and **Table 3** revealed that the highest percentage of radical scavenging inhibition were shown by the methanol extract of black rice variety each for DPPH (89.29±0.139) as well H_2O_2 (80.97±0.091) respectively in a concentration dependent manner with an IC_{50} value of 40 µg/ml (DPPH) compared to the 59.6µg/ml (aq. extract) and 53.1µg/ml (methanol extract) of Joha variety and 49.0 µg/ml (aq. extract) and 45.3 µg/ml (methanol extract) of Bora rice variety. Significant value of 42.6 µg/ml was also obtained for H_2O_2 as compared to its 60.1 µg/ml (aq. extract) and 57.4 µg/ml (methanol extract) of Joha rice and 58.7 µg/ml and 55.2 µg/ml of Bora rice. The studied results were positively correlated with the work done by Zhang MW and co-workers [37] on black rice bran which was considered to be higher due to the presence of anthocyanin pigment. Zhang *et al.,* [36] also reported a related study of total phenolic content and antioxidant activity associated with rice pericarp. It is also remarkably noted that antioxidant activity may vary according the type of the rice variety Gaydou *et al.,* [26]. Although the experiments done for this study includes three rice genotypes of different types. The confirmation of the antioxidant activity of both the extracts can therefore be confirmed for the presence of different chemical constituents present in the extracts which has also been reported to be concomitant with the development of the reducing power [17].

Minimum Inhibitory Concentration: The evaluation of potential minimum inhibitory concentration of the crude rice extracts was done and the MIC of the black rice extract exhibited a value of 119 µg/ml followed by the values of 220µg/ml and 251 µg/ml for Bora and Joha rice varieties.

These values were in accordance to the work of Pornpan *et al.,* [9] on the antibacterial and minimum inhibitory concentration of coloured rice against *S. aureus*. The reported values of the work were however found to be higher than the values obtained in our study. Moreover, the other reported studies on rice have significantly shown an effective activity of its varieties against other tested organism. It has also been shown effective in combating the problem associated with gastritis by suppressing damage to the gastric mucosa by *Helicobater pyroli* in infected Mongolian gerbils [31]. The rice hull smoke extract has been shown to inhibit *S. enterica* serovar Typhimurium *in vitro* in a study reported by Kim *et al.,* [39] that protected an infected mice from death. Presently our study strongly suggested the antibacterial activity of the rice varieties with an increased activity of black rice and anti-staphylococcal activity.

TABLE 2: % INHIBITION OF DPPH BY DIFFERENT CONCENTRATIONS OF EXTRACT AND STANDARD

Sl. No.	Concentration (µg/ml)	%inhibition (water extract)*			%inhibition (methanol extract)*			%inhibition (Standard)*
		Black	Joha	Bora	Black	Joha	Bora	
1	10	35.78±0.098	47.16±0.095	28.42±0.121	25.41±0.139	37.12±.108	44.40±.092	93.48±0.007
2	20	54.51±0.098	51.33±0.095	43.81±0.121	41.63±0.139	48.82±.108	51.01±.092	94.48±0.007
3	40	64.54±0.098	58.70±0.095	62.54±0.121	63.33±0.139	61.88±.108	65.38±.092	94.68±0.007
4	80	68.39±0.098	62.78±0.095	71.40±0.121	77.59±0.139	75.41±.108	72.57±.092	96.15±0.007
5	160	86.28±0.098	84.29±0.095	85.95±0.121	89.29±0.139	87.95±.108	88.13±.092	96.65±0.007

TABLE 3: SHOWING THE % INHIBITION OF H_2O_2 BY DIFFERENT CONCENTRATIONS OF EXTRACTS AND STANDARD

Sl. No	Concentration (µg/ml)	%inhibition (water extract)*			%inhibition (methanol extract)*			%inhibition (Standard)*
		Black	Joha	Bora	Black	Joha	Bora	
1	10	25.54±0.133	9.06±0.185	6.51±0.195	48.78±0.091	15.83±0.162	49.93±0.091	35.75±0.163
2	20	49.55±0.133	44.31±0.185	44.69±0.195	70.11±0.091	48.27±0.162	65.26±0.091	60.40±0.163
3	40	65.24±0.133	63.21±0.185	63.22±0.195	75.35±0.091	59.00±0.162	76.88±0.091	75.99±0.163
4	80	70.02±0.133	68.45±0.185	69.85±0.195	79.44±0.091	62.32±0.162	78.92±0.091	86.84±0.163
5	160	75.73±0.133	74.59±0.185	75.48±0.195	80.97±0.091	78.28±0.162	80.46±0.091	94.39±0.163

CONCLUSION: The findings exhibited a stronger basis of future application of coloured and scented varieties including other rice varieties of North Eastern region which may protect by combating the out coming chronicity associated with the antioxidant stress as well as the infection caused by harmful human pathogens. This way they can also be used as a better supplement for the diseases by bringing them with a newer form of treatment. Also, in addition to developing more nutritious varieties, awareness of the benefits of eating black rice should be raised among rice consumers. The activity obtained by the rice varieties might be due to the synergistic effect of the active compounds that can be purified, isolated and characterized for their further study.

ACKNOWLEDGEMENT: The authors acknowledge the Department of Biotechnology, Government of India for the start-up fund to carry out the study and Assam downtown University for helping in promoting faculty research and Dr. P.P. Baruah (Professor and Head, Department of Botany, Gauhati University, Guwahati, Assam for his invaluable help in the identification of the rice varieties.

CONFLICT OF INTEREST: The authors declared no conflict of interest.

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How to cite this article:
Devi R, Devi B, Unni BG and Garg M: An *in vitro* comparative study on antioxidant, antibacterial and nutraceutical properties of three different coloured scented rice varieties of North- Eastern region. Int J Pharm Sci Res 2017; 8(7): 2968-74.doi: 10.13040/IJPSR.0975-8232.8(7).2968-74.