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# Morphological study, nutritional value and phytochemical estimation of four indigenous rice (*Oryza sativa* L.) varieties of Assam, India

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**Abstract**

India is a major producer and consumer of rice. Assam is one of the top rice-producing states of the country and is situated in the indo-Burma hotspot, it is gifted with exceptionally rich rice diversity. These indigenous rice varieties have unique properties and are cultivated by the local farmers and are only used for the preparation of traditional cousins. In the present study, four indigenous rice varieties namely *Kumol saaul*, *Kola Bora*, *Kola kunkuni Joha* and *Khamti Lahi* have been selected for nutritional profiling and bioactive compounds screening. The biochemical estimation of the nutrients was carried out following the guidelines of the Association of Analytical Chemists International. For phytochemical screening, the methanolic extract of the rice samples was prepared and qualitative as well quantitative tests were performed following standard protocols. The carbohydrate content was higher in *Kumol saaul* (48.3±0.34) and *khamti Lahi* (38.64±0.4) and therefore may be used to prepare breakfast for the farmers while working in the paddy field and to prepare traditional cousin *Tupula vat* respectively. The protein content was highest in *Kola bora* (8.9±0.02) which are used for the preparation of traditional food. Crude lipid, Crude fiber, vitamin and Mineral content also showed significant differences in the four rice varieties. The total anthocyanin content and antioxidant activity were found more in *Kola Bora* (328.26±0.87, 81.45±2.29) and *Kola Kunkuni Joha* (11.90±0.89, 67.34±1.23). Thus, the study clearly showed that these indigenous rice varieties are not only traditionally important but also a good source of nutrients and bioactive compounds. It is expected that, in-depth knowledge of nutrients and other bioactive compounds in the rice varieties may be beneficial for their promotion in global the market as well as germplasm conservation.

## 1. Introduction

Rice is the staple food for over half of the world's population and is considered a leading source in terms of calories (Tong *et al.,* 2019). Rice is the most significant crop cultivated in India in a wide range of agro-ecological situations. Assam, located between 89° 42′ E to 96° E longitude and 24° 8′ N to 28° 2′ N latitude is endowed with exceptionally rich rice diversity along with other floral and faunal diversity. Assam is considered one of the centers of rice origin and has achieved a wide collection of indigenous rice varieties. The variations in soil and climatic conditions of Assam may be the possible factors for the evolution of several indigenous varieties in this region (Deka *et al.,* 2014). Besides, the region is inhabited by a large number of ethnic groups and from time immemorial they have been using some of these indigenous rice varieties by the traditional method of preparation without knowing their health benefits and commercial value in the present-day world.

The four main categories of rice cultivars grown in Assam are *Sali* rice (winter rice), *Boro* rice (summer rice), *Bao* rice (deepwater rice) and *Ahu* rice (autumn rice), having various traits like stickiness, color difference, waxy and non-waxy and specific aroma. Out of these varieties, in the present study, we have selected four indigenous rice varieties namely *Kumol saaul*, *Kola Bora*, *Kola kunkuni Joha* and *Khamti Lahi* which have high cultural demand. *Kumol saaul (Chakua rice grain)* is a kind of *Sali* rice grown in parts of lower Assam. The rice paddy is first parboiled and then de-husked by '*Dhenki*', a traditional foot pounding machine. This rice does not need to be cooked and can be consumed by soaking in water for one to two hours in hot or cold water with milk or curd and jaggery (Figure 1). This rice preparation is very popular in community feasts and

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festivals. *Kola Bora* is black and is a unique variety of waxy rice. It is an Assamese breakfast cereal (*Jolpan*) and is also used for the preparation of *kheers* and *pithas* (cake) during festivals (Figure 2). This cultivar is also used for producing high-class rice beers by the tribes of Assam. *Kola kunkuni Joha* is one of the aromatic indigenous rice varieties of Assam, having superfine kernels, excellent palatability and taste. *Khamti lahi* is a type of sticky rice which are not consumed on daily basis but is used to prepare *tupula vat* by the Tai Khamti and Missing tribes of Assam during festivals and special occasions. The rice is rolled inside *koupaat* (*Phyrinium sp*. Lin.) and then steamed (Figure 3).

Figure 1. Traditional presentation of breakfast cereal *Kumol Saul*

Figure 2. Traditional *Pitha* prepared from *Kola bora* rice

Figure 3. *Tupula bhat* prepared from *Khamti lahi* rice variety

In recent times, people are being more health-conscious and prefer to have selective foods according to their health condition. Rice, being the staple food in a country like India, knowledge on nutrient composition of the different varieties of rice is essential. Kashyap and Mahanta (2016) reported that *Kumol saaul* gives more than 400 Kcal energy (g/100 g) depending on the strain and processing method. According to McKevith and Jarzebowaska (2010), breakfast cereals play an important role in a balanced diet. Black rice is gaining much importance all over the world due to the presence of some unique qualities in it which are beneficial for health. Yawadio *et al.* (2007) considered the black rice varieties more nutritious as they are rich in iron (Fe), zinc (Zn), proteins, and vitamins and possesses antioxidant properties.

Earlier studies have demonstrated the importance of diet in the control of chronic diseases, such as cancer and cardiovascular problems (Birt *et al.,* 2001; Houston, 2005). This could be attributed to the presence of minerals, vitamins, natural antioxidants in these foods (Choi *et al.,* 2007). Several compounds with antioxidant activity have been identified in rice, including phenolic compounds, tocopherols, tocotrienols and g- oryzanol (Iqbal *et al.,* 2005). Although bioactive compounds are found in several foods, variation is observed in the concentration and type of these compounds due to genetic and environmental factors and processing conditions (Kris-Etherton *et al.,* 2002).

Rice, being one of the most produced and consumed cereals in the world, has an important role in the relation between diet and health. However, not much research has been done so far to know the nutrients and bioactive compounds in the indigenous rice varieties of Assam. As a result, the newer generations are unaware of the importance of these indigenous cereal grains and much attracted to junk food. At the same time, due to the introduction of a high yielding hybrid variety of rice, most of the indigenous rice varieties are under threat. Considering the paucity of work related to the nutritional as well as the phytochemical profile of these rice varieties of Assam, India the present study has been carried out. It is expected that the knowledge of the nutritive value as well other bioactive compounds will be helpful to promote the indigenous rice varieties of Assam in the global market and to encourage the local farmers for the cultivation of these varieties on a large scale which may be also helpful to conserve their genetic variability.

## 2. Materials and methods

### *2.1 Sample collection*

Four indigenous rice varieties of Assam were collected from the local farmers (Table 1) based on available literature and information adopting the random sampling method.

### 2.2 *Morphological study*

After collection, the physical observation of the rice grains was done under a stereo zoom microscope (Model

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Table 1. Site of sample collection

| Serial No. | Common name | Area of Collection | Latitude | Longitude |
|---|---|---|---|---|
| 1 | *Kumal saul* | Nalbari, Assam | 26.4446°N | 91.4411° E |
| 2 | *Kola Bora* | Goalpara, Assam | 26.1641° N | 90.6252° E |
| 3 | *Kola Kunkuni Joha* | Nagaon, Assam | 26.3480° N | 92.6838° E |
| 4 | *Khamti Lahi* | Dhemaji, Assam | 27.6087° N | 94.7692° E |

-ZEISS Stereo Discovery.V20) and photographed. The Kernel Length and breadth of ten grains from each sample were determined using a vernier calliper.

*2.3 Biochemical estimation of nutrients*

For biochemical analysis, the rice samples were ground to obtain rice flour which was then stored in separate airtight containers in a cool and dry environment for further analysis. The carbohydrate content in each sample was estimated by using the Anthrone method (Trevelyan and Harrison, 1952). The protein content was measured by using the Lowry method (Lowry *et al.,* 1951). Crude Lipid content was estimated as crude ether extract of the dry material and the procedure to calculate lipid was done as per the official Methods of Association of Analytical Chemists (AOAC) (1990). Crude fiber content was determined using the method described in IS: 10226-1. The Procedure was carried out in the food quality control laboratory, Department of food engineering and technology, Tezpur University, Assam, India. The mineral analysis was done by Atomic Absorption Spectrophotometer (AAS) (Model- Thermo Fisher Scientific AASIce 3500) using Flame Technique in the same department only. For estimation of thiamin (Vitamin B1) and Niacin (Vitamin B3), the method of Okwu and Josiah (2006) was followed.

*2.4 Extract preparation*

For phytochemical estimation, the methanolic extract was prepared. For this, 10 g of powdered rice sample was weighed into a conical flask and 90 mL of pure methanol was added and left for 72 hrs. The mixture was filtered and this methanolic filtrate was concentrated under reduced pressure on a rotatory evaporator at 40°C and then stored at 4°C for further analysis.

*2.5 Test for flavonoids*

A few drops of 20% sodium hydroxide was added to 2 mL of each extract of the rice samples. To this, a few drops of 70% dilute hydrochloric acid was added and colour change was observed. The appearance of yellow colouration which disappears on standing indicates the presence of flavonoids in the sample. The total flavonoid content in all the extracts was determined spectrophotometrically following Pourmorad *et al.* (2006).

*2.6 Test for phenol*

To 2 mL of each extract of the rice samples, 2 mL of 5% aqueous ferric chloride were added. The formation of blue color indicates the presence of phenols in the sample. The total phenolic contents of the extracts were determined by using the Folin Ciocalteu Phenol reagent, following the method described by Singleton and Rossi (1965).

*2.7 Total anthocyanin*

The total anthocyanin content was determined in all the rice samples by the pH - differential method given by Guisti and Wrolstad (2015).

*2.8 Antioxidant activity*

The total antioxidant activity was determined by using DPPH (2,2-diphenyl-1picryl-hydrazyl), described by Sutharut and Sudarat (2012).

*2.9 Statistical analysis*

All the experiments were repeated three times to reduce the experimental error. The results of all the experiments were confirmed by following the statistical method (mean and standard error method). Multiple comparisons were made by one-way analysis of variance (ANOVA). All statistical analyses were performed using MS Excel (2010). Value of $p<0.05$ was considered statistically significant. Post Hoc Tukey HSD was performed and samples belonging to the different subsets were represented by different alphabets and it indicates more variation among them and vice versa.

## 3. Results

The kernel colour of all the rice samples was different. It was yellowish-white in *Kumal saul*, black in *Kola bora*, greenish-white in *Kola kunkuni joha* and Yellowish white in *Khamti Lahi* (Figure 4, 5, 6, and 7). The length and breadth of the rice samples were recorded as (Lenghth×Breadth in cm) 0.72±0.00×0.35±0.01, 0.77±0.01×0.25±0.00, 0.57±0.01×0.23±0.01 and 0.64±0.01×0.3±0.01 in *Kumal Saul*, *Kola bora*, *Kola kunkuni joha* and *Khamti Lahi* respectively but the variation was not statistically significant (Table 2).

Macronutrient analysis revealed the highest

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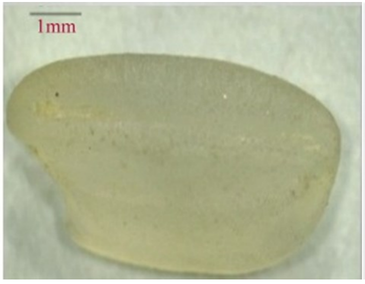


Figure 4. Rice grain of *Kumol Saul*

Figure 5. Rice grain of *Kola bora*

Figure 6. Rice grain of *Kola Kunkuni joha*

Figure 7. Rice grain of *Khamti lahi*

Table 2. Morphological observation of four rice samples

| Parameters | *Kumal saul* | *Kola Bora* | *Kola Kunkuni Joha* | *Khamti Lahi* |
|---|---|---|---|---|
| Kernel colour | Yellowish white | Black | Greenish white | Yellowish white |
| Length (cm) | 0.72±0.00 | 0.77±0.01 | 0.57±0.01 | 0.64±0.01 |
| Breath (cm) | 0.35±0.01 | 0.25±0.00 | 0.23±0.01 | 0.3±0.01 |

Values are expressed as mean±SE, n = 10.

Table 3. Variation in nutrient contents (g/100 g) of the four selected indigenous rice varieties.

| Rice varieties | Carbohydrate | Protein | Crude lipid | Crude fiber |
|---|---|---|---|---|
| *Kumal saul* | $48.3±0.34^a$ | $4.8±0.34^c$ | $0.82±0.04^d$ | $0.43±0.01^d$ |
| *Kola Bora* | $35.05±0.49^c$ | $8.9±0.02^a$ | $4.19±0.11^a$ | $1.34±0.04^a$ |
| *Kola Kunkuni Joha* | $36.1±0.72^c$ | $5.64±0.06^b$ | $1.67±0.10^c$ | $0.51±0.04^c$ |
| *Khamti Lahi* | $38.64±0.4^b$ | $5.8±0.04^b$ | $1.78±0.08^b$ | $0.79±0.03^b$ |

Values are expressed as mean±SE, n = 6. Values with different superscript within the same column are significantly different ($p < 0.05$) between analyses within each sample.

Table 4. Variation in mineral contents (mg/100 g) in four rice varieties.

| Rice varieties | Iron | Zinc | Calcium |
|---|---|---|---|
| *Kumal saul* | $0.86±0.03^c$ | $3.31±0.01^b$ | $14.86±0.01^b$ |
| *Kola Bora* | $1.21±0.01^a$ | $2.67±0.01^c$ | $16.13±0.07^a$ |
| *Kola Kunkuni Joha* | $0.98±0.02^b$ | $2.30±0.02^d$ | $13.16±0.01^c$ |
| *Khamti Lahi* | $0.52±0.01^d$ | $4.53±0.03^a$ | $12.39±0.03^d$ |

Values are expressed as mean±SE, n = 3. Values with different superscript within the same column are significantly different ($p < 0.05$) between analyses within each sample.

carbohydrate content in *Kumal Saul* (48.3±0.34 g/100 g), highest protein, crude lipid and crude fibre in *Kola bora* -8.9±0.02 g/100 g, 4.19±0.11 g/100 g, 1.34±0.04 g/100 g respectively (Table 3). The mineral contents also showed significant variations among the rice samples (Table 4). Iron and calcium content were found highest in *Kola bora* whereas Zinc content was found highest in *Khamti lahi*.

The vitamin Niacin (Vitamin B3) and thiamine (Vitamin B1) were assessed in all four rice samples and are represented in Table 5. After positive qualitative analysis, significant differences were observed in phenolic content and flavonoid contents among the four rice varieties (Table 6). Total phenols, total flavonoids and total anthocyanin contents were recorded highest (73.65± 0.27, 18.68±0.87, 328.26±0.87) in *Kola bora*.

The antioxidant activity in terms of radical scavenging activity during DPPH assay, the percentage of inhibition of various concentrations (25-500 µg/mL) and ascorbic acid standard (5-50 µg/mL) were calculated and recorded as shown in Table 7. Following DPPH assay regression analysis showed that phenolic compounds contribute to about 74% ($r^2 = 0.744$) of radical scavenging activity. Similarly, flavonoids contribute to 85% ($r^2 = 0.852$) of antioxidant activity in the studied rice samples.

Table 5. Variation in vitamin contents (mg/100 g) in four rice varieties

| Rice varieties | Thiamin | Niacin |
|---|---|---|
| *Kumal saul* | $0.34±0.07^c$ | $3.52±0.11^d$ |
| *Kola Bora* | $0.52±0.12^a$ | $5.01±0.13^a$ |
| *Kola Kunkuni Joha* | $0.44±0.13^b$ | $4.71±0.09^b$ |
| *Khamti Lahi* | $0.35±0.07^c$ | $3.69±0.11^c$ |

Values are expressed as mean±SE, n = 3. Values with different superscript within the same column are significantly different ($p < 0.05$) between analyses within each sample.

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Table 6. A comparison of total phenolic content (mg GAE/g dry weight), total flavonoid content (mg QE/100 g dry weight) and total anthocyanin content (cyanidin-3-glucoside equivalent mg/100 g) in four rice varieties.

| Rice varieties | Total phenols | Total flavonoid | Total Anthocyanin content |
|---|---|---|---|
| *Kumal saul* | 18.41±0.13[d] | 6.13±0.34[d] | 8.35±0.98[c] |
| *Kola Bora* | 73.65±0.27[a] | 18.68±0.87[a] | 328.26±0.87[a] |
| *Kola Kunkuni Joha* | 33.65±0.28[b] | 11.29±0.28[b] | 11.90±0.89[b] |
| *Khamti Lahi* | 21.34±0.18[c] | 7.19±0.56[c] | 6.71±0.69[d] |

Values are expressed as mean±SE, n = 3. Values with different superscript within the same column are significantly different ($p < 0.05$) between analyses within each sample.

Table 7. Variation in radical scavenging activity (%) in four rice varieties

| Rice varieties | Radical scavenging activity (%) |
|---|---|
| *Kumal saul* | 13.23±1.12[d] |
| *Kola Bora* | 81.45±2.29[a] |
| *Kola Kunkuni Joha* | 67.34±1.23[b] |
| *Khamti Lahi* | 17.18±1.11[c] |

Values are expressed as mean±SE, n = 3. Values with different superscript within the same column are significantly different ($p < 0.05$) between analyses within each sample.

## 4. Discussion

Nutritionally rice is one of the major cereal grains and is consumed by more than half of the world's population. However, the quantitative estimation of nutrients in rice shows significant variation according to the genotype, geographical location, cultivation method as well as processing method to prepare the consumable form of rice grain. In the present study, all four varieties of rice samples were de-husked by using traditional *Dhenki* except *Kumol Saul* needs parboiling prior to de-husking. In Assam, rice occupies a specific position in culture and tradition as it is the main crop of the region. The region is also endowed with a large number of indigenous rice varieties due to unique geo-climatic conditions.

Carbohydrate is the major nutrient content in rice. In the present study, it was found that that the carbohydrate content varies between 35.05-48.3 mg/100 g in the four rice varieties, which is very much similar to the findings of Vora *et al.* (2015). *Kumol Saul* recorded the maximum carbohydrate content (48.3%) and therefore may be traditionally *Kumol Saul* is a popular breakfast cereal in rural places of Assam and consumed by the farmers while working in fields to give the energy boost to the body which is required for laborious work. Although the protein, crude lipid and crude fibre content in *kumol Saul* are comparatively lower than the other rice varieties, the values obtained for these parameters are nutritionally satisfactory. On the other hand, *Kumal Saul* is a rich source of Fe, Zn and Ca as well as thiamine and niacin vitamins. Thus, *Kumol Saul* can be promoted as a healthy fast food for the youngsters as its preparation needs no cooking. Prior to consumption *Kumol Saul* has to be soaked in hot water for two hours and can be taken with curd and jiggery. But as the radical scavenging activity (13.32%) is not much higher in *Kumol Saul* and is very rich in carbohydrate content it is not recommended as good food for diabetics and heart patients and it also increases the tendency for weight gain (Saleh *et al.,* 2019).

The protein content was found highest in *kola bora* which is also sticky and used to prepare traditional cakes and rice beer during festivals. The carbohydrate content was lowest in *kola bora* among the four studied rice samples whereas the crude lipid and crude fiber contents were highest. Although lipid content in rice is not higher as the carbohydrate and protein, it plays an important role as a component of various essential fatty acids (Hemavathy and Prabhakar, 1987). Crude fibre regulates digestion, improves bowel movements and boost metabolism (Lovergrove *et al.,* 2006). This rice variety is also a good source of minerals like iron and calcium as well as vitamins niacin and thiamine. The best part of *kola bora* is that it has the highest amount of total phenols, flavonoids and anthocyanin content as reported by others workers in different varieties of black rice (Zhang *et al.,* 2006) and thereby the radical scavenging activity is 81.45%. These phytochemical compounds are usually accumulated in the pericarp or bran of rice kernels (Sutharut and Sudarat, 2012) and hence finer processing of the rice grains may reduce the bioactive compounds as like in white rice (Sompong *et al.,* 2011). Earlier studies have shown that pigment of fruits, vegetables and coloured rice have important roles in reducing the risk of cancer and other chronic diseases because of their free radicals scavenging capacities (Wang and Stoner, 2008; Elisia and Kits, 2008). In the recent period, research on bioactive compounds of natural origin is gaining importance because of their disease-fighting properties in disorders such as lowering the risk of cancer formation, coronary heart disease and lowering of cholesterol (Akihisa *et al*., 2000; Xu *et al.,* 2001; Rohrer and Seibenmorgen, 2004). Thus, *kola bora* can be considered as medicinally important rice which can be further used to prepare commercial products having antioxidant properties. *Kola Kunkuni joha* rice variety is famous for its specific aroma. At the same time, the rice variety is a good source of nutrients like carbohydrates (36.1 g/100 g), protein (5.64g/100 gm), crude lipid (1.67 g/100 g), minerals and vitamins as reported by Verma and Srivastav (2017) in other aromatic Indian variety of rice. The present findings suggest that *Kola Kunkuni joha* rice can be a good option for consumption on daily basis. The study also shows that *Kola Kunkuni joha* contains a considerable amount of phenols, flavonoids and anthocyanin which has a close consortium with the findings of Rahman and Eswariah (2017) for aromatic indigenous rice of Assam. Hence, the rice has 67.34% radical scavenging activity next to *kola bora*.

*Khamti Lahi* rice is not consumed on daily basis but is used to prepare *tupula vat* by the Tai and Missing tribes of Assam specifically during festivals. It may be due to the fact that carbohydrate content is more in this rice variety compared to other rice varieties and as it is prepared in steam there are no chances to lose carbohydrates during cooking. Daily consumption of this kind of rice may lead to obesity and an increased risk of diabetes. The rice contains an average amount of protein and crude fibre like other common white rice varieties (Godber and Juliano, 2004). Crude lipid content was found more in this rice variety which indicates that it helps in bowel movement and boost metabolism (Kolahdouzan *et al.,* 2013). The iron content was very low, calcium content was medium but a higher amount of Zn is present in this rice sample. Zn plays an important role in various metabolic activities of the body as it is a part of many enzymes, it is present in insulin, involved in making genetic material and proteins, immunity, vitamin A transport, wound healing, making sperm, normal fetal development. The thiamine and niacin content was average as reported in other rice varieties (Verma, 2011). Although the total phenols, flavonoids and anthocyanin contents were more in *Khamti lahi* rice than the *kumol saul*, however, the values are comparatively less than the other pigmented and aromatic rice varieties and radical scavenging activity is only 17.18%. But the unique process of preparation of this rice in *koupat* which gives a specific aroma to the cooked rice during steaming makes the rice extraordinary.

Thus the present study suggests that the indigenous rice varieties along with being traditionally important these rice varieties are also a rich source of nutrients and have medicinal importance as said by local people. *Kumol Saul,* the instant cereal breakfast is a rice source of carbohydrates along with other nutrients. *Kola bora* is not only nutritionally rich but also possesses high radical scavenging activity making it important medicinal rice. *Kola Kunkuni joha* is not only famous for its specific aroma but biochemical analysis suggests that it can be consumed on daily basis. *Khamti lahi* is not exceptionally rich rice in nutrient contents and bioactive compounds but holds an average of all biochemical components. But the specific preparation method of this rice variety during festivals makes it always an exception.

## 5. Conclusion

Lack of knowledge is one of the major hurdles of losing some unique gifts of nature including rice. Because of specific geo-climatic conditions, Assam holds a good number of indigenous rice verities. Being agriculture an important occupation, rice also occupies an important position in the culture and tradition of Assamese people. Because of the introduction of many new high yielding hybrid rice varieties, the cultivation of indigenous rice varieties has lost its importance. But once the unique properties and health benefits of these rice varieties can be established, their demand in the market will be increased. It is expected that this will encourage the local farmers to cultivate more indigenous rice varieties with advanced techniques bringing economic empowerment to rural Assam.

## Conflict of interest

The authors declare no conflict of interest.

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