AN INTEGRATED APPROACH ON STUDY OF NUTRITIONAL AND IN VITRO ANTI CANCEROUS PROPERTIES OF TRADITIONAL PIGMENTED RICES AGAINST MCF7 CELL LINE

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Abstract

Rice is one of the most important cereals and more than two lakh varieties of rice have been grown commercially throughout the world and it is a staple food in Asian countries. Hence, the present study is intended to evaluate the medicinal properties of the traditional varieties especially pigmented rice such as brown rice, black kavuni and sivappu kavuni and assessed the cell toxicity effect on the breast cancer cell line MCF-7. In this study, a concentration-dependent scavenging activity was observed in selected rice samples with a maximum activity of 96% had been achieved which stepped into anti-tumor activities by in vivo, and to develop the drug for to inhibit the metabolic activities of cancer cells.

Keywords: Brown Rice, Black Kavuni Rice, Sivappu Kavuni Rice, cell toxicity, anti-tumor activity, MCF 7 Cell line

INTRODUCTION

Rice is the princess among the cereals. It is the life driven source for half of population in India and 2004 was announced as the international year of rice by the United Nations. Rice is the edible starchy grain and staple food. Since historical era, consuming rice as the food and cultivating more than 2000 types of rice varieties have been one of the rituals in different parts of the world. It is not only the source of nutrient but also possesses lot of medicinal properties those were proven in Ayurveda. In the roots date back thousands of year, Indian traditional system of treatment mentioned the term, Navarakizhi which is one of the ore exotic treatments offered with rice by practitioners.

The medicinal rice values are documented in the Charaka Samhita (700BC) and the Sambhita (400BC) (Savitha and Ushakumari, 2015). The pigmented rice varieties has flavonoid pigments such as anthocyanin and procyaninins which reduce the atherosclerotic plaque formation, inhibit aldose reactivity, decrease hyperlipidaemia and suppress cancer cell (Wannisa Vichit and Nisakorn Saewan, 2015). Based on the region where it is planted, the rice differs in composition of favourable sources like phenolic, vitamins, and antioxidants. Especially, pigmented rice varieties such as brown rice, red rice, and black rice contain enriched nutritional qualities and serve as a good source of antioxidant. In the technology driven world, an instant healthy life is the demand of mankind. Food with natural antioxidant and polyphenols to reduce the oxidation process occurring in the cells is the need of the hour (Norliza et al., 2017). Li et al., 2011 reported that anthocyanin plays a major role in reducing cholesterol and prevent the cardiovascular disease, it also possesses antidiabetic, anticancer, anti-inflammatory and antimicrobial properties. Poonindin-3-glucoside and cyanindin-3-glucoside, the major compounds are involved in actions of inhibition/penetration of cells on different cancer are present in black rice. The carotenoids are the compounds that used to decrease the formation of cancer cell and heart disease (Saravavan Ponnappan et al., 2017).

Breast cancer states 87,090 lives in India every year and it accounts for 27 % of all cancers in women. It is impacting 21 lakh women globally per annum and 1,62,468 new cases are being added in India every year. The incidence rates in India began to rise in early thirties and high in the age group of 50
– 64 years. The disease marked itself in the form of a painless lump in the breast, which can aggravate to pain, swelling and nipple abnormalities. Efficient and affordable treatment is the right for all women. But, in various cultures, women suffer from gender disparity when it comes to accessing health services. In poor countries, there is the additional burden of a lack of admission to treat with many, and the high cost of medicines. The average cost of treatment for breast cancer through a private practitioner would be Rs 5-6 lakh, including investigations, surgery and radiotherapy. Keeping view on the traditional practices, aligning with food as the medicine, the present study is intended to evaluate the nutritional properties of traditional pigmented rice varieties of Tamil Nadu and assess anti-cancer activity for breast cancer.

**MATERIALS AND METHODS**

**Collection of sample**
Pigmented rice varieties such as sivappu kavuni (red), black kavuni, and brown rice were collected from Musiri, Ariyalur, and Thiruthuraipoondi.

**Sample preparation**
All selected rice sample was dried under sunlight and made into flour using pulvarizer machine. The following physiochemical properties were estimated.

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<th>S.No.</th>
<th>Physiochemical property</th>
<th>Method</th>
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<tr>
<td>1.</td>
<td>Moisture</td>
<td>AOAC (1990)</td>
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<td>2.</td>
<td>Protein</td>
<td>Lowry’s (1951)</td>
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<td>3.</td>
<td>Carbohydrate</td>
<td>Anthrone (1962)</td>
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<td>5.</td>
<td>Fat, FFA</td>
<td>AOAC (1990)</td>
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**Anti-oxidant activity by DPPH Method (Lin and Yen, 1999)**
Rice samples were grained well with distilled water, extracted thrice with equal amount of 99.9% ethanol and the dried samples were used for anti-oxidant activity. Each sample was taken at the concentrations of 2, 4, 6, 8 and 10 mg/ml and 1 ml of a 0.3 mm DPPH solution was added to each sample at different concentrations and allowed to react at room temperature. After 30 min, the absorbance (Ab) values were measured at 518 nm and converted into the percentage of antioxidant activity using the following equation

Scavenging capacity% = 100 – [(Ab of the sample - Ab of blank) × 100/Ab of control]

**In vitro anticancer activity assay**
To study the cytotoxic effect of human oral carcinoma cell lines (KB) were procured from National Centre For Cell Science (NCCS), Pune, India, and the cell lines were maintained in a flask containing Dulbecco’s modified eagle medium (DMEM) and supplemented with 10% (v/v) FBS, 100 units/ml penicillin, and 100 mg/ml streptomycin. The cells were incubated in a 37 °C and 5.0% CO₂ atmosphere incubator.

**MTT assay (Mosmann, 1983)**
Cytotoxic activity of extracts on KB cells was assessed based on mitochondrial dehydrogenase activity in living cells. 3-(4, 5-dimethylthiazol-2-yl) -2, 5-diphenyltetrazolium bromide dye reduction assay was carried out to find out the cytotoxic effect of the bioactive compound at various concentrations. 1 × 10³ cells were plated onto 96-well plates, after 24 h incubation, the cells were treated with different concentrations of sample extracts and the cells were incubated for 24 hours at 37 °C in a humidified incubator. After incubation at 5% CO₂, 100 µL of MTT (5 mg/ml in PBS) was added to each well, further, the plates were incubated for four hours at 37°C. The resulting formazan was dissolved in 100 µL of DMSO and the absorbance of the solution was read at 595 nm using a multiwall plate reader.
(Tecan Multimode Reader, Austria). All determinations were carried out in triplicate. Concentrations of test sample showing a 50% reduction in cell viability (i.e., IC 50 values) were then calculated.

RESULTS & DISCUSSION

Physiochemical Parameters

Physicochemical properties of the selected rice varieties were estimated to determine their appropriate uses.

Moisture content in rice sample

Moisture content plays a significant role in determining the shelf life (Webb, 1985) of food commodities. Moisture is dependent on the genetic makeup of varieties, agronomic and climatic condition as well as processing conditions (Butt et al., 1997). The Moisture content of the samples was presented in Fig.1. The low moisture content was found in sample 1 brown rice (9.5±0.2%) whereas the highest moisture content was observed in sample 2 and 3 (13.4±0.5). The result of the present study was well supported by the findings of several researchers who found the moisture content variation from 7 to 11%. Pusa Basmati 1 and Swarnamukhi (NLR 145) varieties showed the highest percentage moisture content (11.6%), While Bhuban variety (7.13%) contained the lowest percentage of moisture content at 0% polishing; Pooja and Bhuban contained highest moisture as 11.3% and 7.1% respectively at 5 % polishing; KMP 101 and Bhuban displayed the highest and lowest moisture percentage of 11.3 and 7.0% respectively at 10% polishing (Awan, 1996). The high percentage of moisture content affects the milling characteristics and the taste of cooked rice (Xheng and Lan, 2006). Anjum et al., 2007 found moisture content ranging from 8.61 to 11.08% in different milling fractions of rice and the highest moisture content was found in brown rice of different varieties. Ebuexi and Oyewole (2007) reported that the moisture content of rice also affects its storage. It follows that Bhuban rice variety may have a longer shelf life compared to the other rice varieties due to the lower moisture content at all polishing levels. The variation observed in moisture content among rice varieties may be attributed to differences in the genetic makeup as well as climatic conditions. The results suggested that the moisture content found in the present study is well within safe limit.

Ash content of the rice samples

Ash content refers the total amounts of minerals present in the food. Ash content of the samples was given in Fig.2. Low ash content was found in brown rice (1.72±0.02%) whereas the highest ash content was observed in black kavuni rice as (13.3±0.5) and sivappu kavuni rice as (13.8±0.8). The results of the present study are well supported with the results of several researchers (Oko, A. O. et al., 2015) who found that ash content from 0.05 -2.0 % with a mean of 0.99±0.42 % in rice varieties of Canada had shown the highest amount of ash, followed by Mass (I), Chinereugo and Eziccihi, while Faro15 (I), Awilo, E4077, E4314 and Mass (II) had the least values. Silifat Aijoke Sanni et al., 2005 found that cooked rice samples were uniform in ash content with ranged from 0.37 - 0.43.

Fig. 2 Ash content of the sample
The variations observed in ash content among rice varieties might be attributed due to the differences in the genetic makeup as well as climatic conditions.

**Protein content of the sample**

The maximum protein content, 8.9±0.03 % was observed in red kavuni and brown rice possessed a minimum of 6.7±3.6 (Fig.3). Awan, 1996 reported a range of 7.38 to 8.13% protein in different Pakistani varieties. These levels of protein in rice are very essential as protein from the basic building blocks for cell and tissue repairs in the body.

Fig.3 Estimated protein content in the sample

Rice, a major staple food in the world trade, provides 20% of the world’s dietary energy supply (FAO/IRRI 2006). The protein is highly digestible with excellent biological value and protein efficiency ratio owing to the presence of higher concentration (~4%) of lysine (FAO/WHO 1998). The nutritional values of rice generally vary based on different varieties, soil fertility, fertilizer application and other environmental conditions.

In a study conducted by Guha and Mitra (1963), with 74 Indian varieties, a similar range of 6.7 to 11% protein in brown rice was observed and indigenous cultivators of the North Eastern hill states of India possess high protein content with a range of 6.14 to 12.07%. (Devi et al., 2008 A; Premila Devi et al., 2010). Ahmed et al., (1998) reported that the crude protein content of nine aromatic rice cultivators are ranging from 9.17 to 11.77%. Protein and carbohydrate content of the formulated rice products from different traditional varieties were tested with different genotypes would help the nutritionist to assess the protein intake and deficiency of protein among the people of rice consuming countries.

**Carbohydrate content of the sample**

Rice is the starchy staple food and a major source of carbohydrates. The carbohydrate content in the brown rice CO43 sample showed a maximum of 64±0.2% to a minimum amount in black kavuni rice and sivappu kavuni rice (54±0.2% and 35.9±0.3%) (fig. 4). The high per cent carbohydrate content of the rice varieties signifies a high level of starch and it shows that rice is a good source of energy.

Fig.4 Estimated carbohydrate content in sample
The present study has revealed that brown rice, black kavuni and sivappu kavuni with considerable amount of carbohydrate and protein are the good source of energy for body maintenance and also repair and replacement of worn-out or damaged tissues.

**FAT content of the sample**

The fat content of rice varieties plays a significant role in food commodities. The low fat content was found in the sample of brown rice (1.02 ±0.5) and high fat content present in black kavuni rice (1.75±0.05) and sivappu kavuni rice (1.50±0.01) as given in Fig.5. The present study result was aligned with the work done by Oko A. et al., 2012 who found that fat content ranged between 0.50 5.50 %, with a mean value of 1.90 ± 1.03 %. Zubair et al., 2012 evaluated the Fat content was found higher in Miniket parboiled milled rice (0.37%) than that of TM-1 and TM-2 (0.24% and 0.26%, respectively). Thus BRRI-28 was found more nutrients dense (higher fat, ash, protein and fibre content) than the Miniket rice of TM-1 and TM-2.

**The Fiber content of the sample**

As fibre in the food used for the good digestion and free from constipation, it is considered to be the significant factor of any food. Fibre content differs based on the genetic makeup and agronomic varieties of the species. In the present study, the high amount of fibre was present in the sample of black kavuni rice (1.58±0.03), the moderate level in brown rice as (0.95±0.01) and low range in sivappu kavuni (0.7±0.01) as showed in Fig.6.

**Fig.5 Estimated fat content of the sample**

**Fig.6 Estimated fiber content of the sample**
This result was well supported with the report of Zubair et al., 2015 who found crude fibre content in miniket brown rice as 0.78 \%, while it was 0.26 \% in miniket milled rice. They also reported that crude fibre content was found slightly higher in the miniket parboiled milled rice (0.26 \%) than that of TM-1 and TM-2 varieties (0.22 \% and 20 \% respectively). Shweta bhosale and Vijayalakshmi, 2015 observed the amount of crude fibre (7.85 and 4.96 g), insoluble dietary fibre (21.17 and 13.10), soluble dietary fibre (2.17 and 1.80), total dietary fibre (23.34 and14.90) in stabilized and probiotic treated rice bran sample.

**FFA content of the sample**

Rice lipids, are mainly in the form of triacylglycerides, which are classified into free lipids and bound lipids (Morrison, 1998 and Zhou et al.,1999). FFA of brown rice, black kavuni and sivappu kavuni was estimated as 3.0±0.05, 3.38 ± 0.05 and 3.90±0.02 respectively in the present study as plated in Fig.7.

Lemos 2000, revealed that fatty acid (FA) fraction, like mono- and polyunsaturated fatty acids (PUFAs) such as palmitic acid (21–26\%), linoleic acid (31–33\%), and oleic acid (37–42\%) are the predominant compounds found in rice bran and its considered a healthy food which prevents the cardiac related diseases. Enochain et al., (1981) reported that below 10 percent FFA is acceptable for human consumption. Hence, the present study showed that the fatty acids in selected rice varieties has acceptable amount, which could be consume by human beings.

**In vitro antioxidant activity**

In this present study, we have evaluated the free radical scavenger activity of rice samples and a concentration-dependent scavenging activity was observed in ethanolic extract of rice samples with a maximum activity of 98 \% in the sivappu kavuni rice, 96\% in black kavuni rice, 92\% in CO43 rice (fig.8). The result shows that the rice samples contain antioxidant compounds such as polyphenols, flavonoids, and phenolic compounds which are responsible for inhibiting or preventing
the deleterious consequences of oxidative stress. In this present study, brown rice showed highest oxidative activity due to the presence of an elevated amount of flavonoid which might be used to cure degenerative diseases such as coronary heart diseases, atherosclerosis, diabetics, ageing and cancer (Finkel et al., 2000; Ito et al., 1986; Safer et al., 1999; Thompson et al., 1998b and Thompson et al., 1998).

**Fig.8 Antioxidant activity of the sample**

Kris Etherton et al. (2002) analyzed that the concentration of total phenolics in the grain has been positively associated with the antioxidant activity and has potential beneficial effects such as reduction of oxidative stress, aid in the prevention of cancer in the control of blood lipids and related diseases, help in the prevention of cardiovascular problems, and in the prevention of the complication of diabetics. Bakiyalakshmi and Boominathan (2014), reported that the crude extract of Navara rice and brown rice contain significantly high polyphenolic compounds with superior antioxidant activity. Jae-Sung Lee et al., 2011 estimated antioxidant activity of rice ranged from 3.67 to 20.5 mg/ml. Chatchawan chotimarkorna et al., (2008) assessed the total phenolic and flavonoid contents, gamma-oryzanols, tocopherols and tocotrienols isomer contents of rice brown rice with antioxidant properties.

The present study examined and revealed the extracts of the brown rice, black kavuni rice and sivappu kavuni rice possess the elevated amount of radical scavenging property.

**Study of cytotoxicity**

The antiproliferative activity of the bran extracts of black, red, and brown rice (25–400 µg/ mL) was evaluated against the breast cancer cell lines MCF-7. The MTT assay indicated that black, red, and brown rice bran reduced the viability of MCF-7 cells. The present study showed that the lethal concentration of the samples showed IC 50 concentration whereas brown rice at the level of 100.12µg/ml, sivappu kavuni of 129.1 µg/ml and the black kavuni at the level of 117.43 µg/ml. The IC50 values differ significantly (p < 0.05) among the different pigmented rice bran. It has been reported that the cytotoxic activity of rice bran gave above 85% cytotoxicity at 100ppm, moderate activity ranging between 50 -85% while low activity was below 50%. Methanol extract of brown rice, black kavuni and sivappu kavuni rice exhibited the most cytotoxic effect against all cell lines used in this study as showed in **fig.12**. Noha S. Khalifa et al., 2013 and Thind et al. (2008) experienced the effect of Taberna extract on the breast (MCF-7) and colon cancer (HT-29) cell lines. When our data were compared with their results we found that the methanolic extract of Tabernaemontana in our case gave higher cytotoxic effect with both HT-29 and CF-7 cells cytotoxic effect with both HT-29 and MCF-7 cells. MCF-7 Cells on brown rice, black kavuni and sivappu kavuni showed the cytotoxicity at the concentration of IC50 as 100.12µg/ml, 129.1 µg/ml and 117.43 µg/ml respectively. (fig.12-15)

**Fig.12.Cytotoxic activity of the sample**
Fig. 13 Cytotoxic activity of brown rice sample

Cytotoxic activity of sample-1 on MCF-7 cells

Fig. 14 Cytotoxic activity of black rice sample

Cytotoxic activity of sample-2 on MCF-7 cells

Fig. 15 Cytotoxic activity of sivappu rice sample
Conclusion

Rice (Oryza Sativa L.) is the grain of life for most of the Asians as stable food. Our traditional rice is not a mere source of energy and Diabetics. It is a complete solution for sustainable health. After the invention of high yielding varieties and hybrids, the traditional rice varieties are eroded and lost their identity. Now a days, the term traditional and organic are gaining more attention. It brings to expect the orderly constructed traditional and indigenous practices are inbuilt with science and technology. Breast cancer among young women generations is one of the major causes of the modernised environment with instant solution for all. Hence, the present study is intended to investigate the nutritional properties of pigmented traditional rice varieties of Tamil Nadu such as brown, black and sivappu rice with the underlining principle, Food as a Medicine. Moisture, carbohydrate, protein, fat, FFA, fiber content of rice varieties were estimated and the results revealed that the pigmented rice varieties are the good source of nutrition. The ethanolic extract of rice samples sivappu kavuni, brown and kavuni rice showed the maximum antioxidant activity of 98%, 96% and 92% respectively. Brown rice exhibited at the level of 100.12µg/ml, sivappu kavuni of 129.1 µg/ml and the black kavuni 117.43 µg/ml of cytotoxicity activity. The results expose the values of traditional practices and further studies would evaluate and brings the sustainable solution to quit breast cancer with the principle, Food as a medicine.

ACKNOWLEDGEMENT

The authors are grateful to thank Scigene Research Laboratory, Periyar Manniammai University Campus, Thanjavur, TamilNadu for providing necessary laboratory facilities to complete this manuscript.

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Saravanan Ponnappan1, Arun Thangavel1 & Omprakash Sahu1(2017)-Anthocyanin, Lutein, Polyphenol Contents and Antioxidant Activity of Black, Red and White Pigmented Rice Varieties, *Food Science and Nutrition Studies* ISSN 2573-1661 (Print) ISSN 2573-167X (Online) Vol. 1, No. 1, 2017


