RESEARCH ARTICLE

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Phytochemical, Antioxidant and Nutrient Analysis of Medicinal Rice (*Oryza Sativa L.*) Varieties Found in South India

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Ten medicinal rice cultivars collected from South Indian states were investigated and compared for their phytochemical composition, nutrient content, antioxidant activity and reducing power. From the phytochemical analysis, Kullakar showed the highest flavonoid (176 ± 6.12 μg/mL) content, whereas Njavara yellow had the highest phenol (152 ± 3.80 μg/mL) content. The antioxidant properties and reducing power of the rice varieties were found out. Ambimohar and Njavara yellow showed the highest reducing power and Ambimohar had the highest antioxidant property. The total protein content was high in Njavara yellow ($10.92 \pm 0.28\%$). Hakkalasali, Njavara black and Chennellu showed the highest carbohydrate content ($74.5 \pm 2.65\%$). The medicinal rice Kullakar showed high thiamine content (0.53 ± 0.01 mg/100 g), while the riboflavin and niacin content was high in Karikalaveya (0.08 ± 0.01 mg/100 g and 16 ± 0.58 mg/100 g respectively). The present study shows that the ten medicinal rice cultivars possess phytochemical, anti-oxidants and nutrient content which is at par with or higher than Njavara.

Keywords: Medicinal Rice, Njavara, Pytochemicals, Antioxidant Activity, Radical Scavenging Activity.

1. INTRODUCTION

Rice is an easily digestible fine cereal used as staple food by large part of the world population. Rice is also a good source of insoluble fibre, protein and vitamins. In addition to the fibre, protein and vitamins, pigmented rice varieties promote human health because they contain antioxidative compounds that have the ability to inhibit the formation of reactive oxygen species.[1] Antioxidant activity of rice is associated with the presence of phenolic compounds, tocopherols, tocotrienols and gamma oryzanol.[2] Ancient ayurvedic literatures mention the use of rice for curing various neuro-muscular, digestive and respiratory illnesses.[3] Tricin and rare flavonolignans present in Njavara possesses antioxidant and antiinflamatory properties.[4] These results support with the privileged use of Njavara in Ayurveda. Certain varieties of rice are being used by traditional healers to cure various maladies including cancer (http://knol.google.com/k/pankaj-oudhia/leucoderma-and-medicinal-rice/3nerdtj3s9l79/4#). Rice is processed for storage by removing the outer layers leaving only the endosperm, preventing the rice from becoming rancid.[5] The rice endosperm contains only starch and protein bodies but it lacks many essential nutrients like carotenoids.[6] The medicinal property of the rice is preserved by using only dehusked rice. In India, medicinal rice varieties are being used in Kerala, Karnataka, Tamil Nadu, Madhya Pradesh, Uttar Pradesh, the Western Ghats, and Himachal Pradesh to treat skin diseases, fever, paralysis, rheumatism, leucoderma, diabetes and other disorders (http://www2.bioversityinternational.org/publications/pgrnewsletter/article.asp?id_article=11&id_issue=122). It is also used as a health tonic.[7]

The anticancerous and anti-oxidative properties of pigmented rice varieties had been studied by Nam et al. and the results showed remarkable anti-oxidative, anti-tumor-promoting and anticarcinogenic activities on mammalian cells.[1] Another investigation revealed that brown rice is rich in phenols that interfere with the colony-forming ability of breast and colon cancer cells.[8] Germinated brown rice possesses certain growth factors which are involved in regulating the blood sugar level (http://www.ayurvedictalk.com/germinated-BROWN-rice-found-to-have-medicinal-value-for-diabetics/795/).

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Whole rice possesses cholesterol lowering properties due to the presence of gamma oryzanol (http://www.botanical-online.com/english/medicinalpropertiesrice.htm). The medicinal properties of Indian rice varieties have not been subjected to proper scientific investigation. The major works on medicinal rice in India were carried out on the medicinal rice Njavara, grown in the South Indian state of Kerala.

Our main objective of this study is to investigate phytochemicals, nutrients, total antioxidant activity and reducing power of the medicinal rice varieties collected from different parts of South India.

2. MATERIALS AND METHODS

2.1. Chemicals and Instruments

Gallic acid (GAE), Sodium carbonate, aluminium chloride, sodium nitrate, sodium hydroxide, quercetin (QE), potassium dichromate, potassium permanganate, hydrogen peroxide, sodium sulphate, potassium cyanide, copper sulphate, sodium tartrate, sodium dodecyl sulphate, anthrone, sulphuric acid, potassium ferricyanide, ammonium molybdate, sodium phosphate, trichloroacetic acid (TCA), ferric chloride were purchased from HiMedia, Bombay, India. Folin-Ciocalteu reagent was purchased from Spectrum, Cochin, India. All the chemicals used including the solvents, were of analytical grade. Spectrophotometric measurements were carried out using Systronics Double Beam UV-VIS Spectrophotometer (M2202).

2.2. Collection of Samples

The paddy samples were obtained from 'Sahaja Samrudha', Bangalore, INDIA which was collected from different regions of Kerala, Karnataka and Tamil Nadu. The list of paddy samples used in this study is given in Table I and the images are shown in Figure 1.

2.3. Preparation of Aqueous Extract

The powdered whole paddy samples (1 g each) were extracted in 10 ml of distilled water for 4 hours at 80 °C in a water bath. After four hours it was filtered through muslin cloth several times and the extract was concentrated to make the final volume one fourth of the original volume. It was then centrifuged at 5000 rpm for 15 min. The supernatant was collected and stored at 4 °C in air tight bottles for further studies.

Table I. List of Medicinal rice cultivars used in this study.

S/N	Cultivar name	Location
1.	Joddubirunellu	Karnataka
2.	Ambimohar	Karnataka
3.	Karikalaveya	Karnataka
4.	Kagisali	Karnataka
5.	Kari gajivile	Karnataka
6.	Hakkalasali	Karnataka
7.	Njavara black	Kerala
8.	Njavara yellow	Kerala
9.	Chennellu	Kerala
10.	Kullakar	Tamil Nadu

2.4. Estimation of Total Phenolics, and Flavonoids

The amount of total phenolics was carried out by Folin-Ciocalteu's reagent using GAE as a standard.[9] The aqueous extracts (0.1 mL) were made up to 1 mL using distilled water and 0.8 mL of Folin–Ciocalteu reagent was added. After 3 min, 2 mL of 7.5% sodium carbonate solution was added. The final mixture was diluted to 7 mL using deionized water. The mixtures were kept in dark at 50 °C for 2 hours and the absorbance was measured at 765 nm. The results were expressed as mg GAE/100 g dry weight.

Total flavonoid content was determined using aluminium chloride calorimetric method using QE as a standard.[10] The aqueous extract (0.1 ml) was added with 0.3 mL of distilled water followed by 0.03 mL of 5% sodium nitrate. After 5 min at 25 °C, 0.03 mL of 10% aluminium chloride was added to the reaction mixture. Further, after 5 min, 0.2 mL of 1 mM Sodium hydroxide was added. The reaction mixture was diluted to 1 mL with distilled water and the absorbance was measured at 510 nm. The results were expressed as mg QE/100 g dry weight.

2.5. Estimation of Total Proteins and Carbohydrates

Total proteins present in the rice extract were estimated using the Folin-Ciocalteu and alkaline copper sulphate reagent.[11] The extract (0.1 mL) was made up to 1 mL using distilled water. It was added with 5 mL of alkaline copper sulphate reagent. The solution was allowed to stand for 10 min after thorough mixing. Then 0.5 mL of Folin-Ciocalteu reagent was added, mixed well and incubated in the dark for 30 min. The blue coloured solution was measured for its absorbance at 660 nm.

Total carbohydrate was estimated using anthrone reagent.[12] The extract (0.1 mL) was made up to 1 mL using distilled water. It was added with 4 mL of anthrone reagent and heated for 8 min in a boiling water bath. The heated mixture was cooled rapidly. The greenish solution was measured for absorbance at 630 nm.

2.6. Estimation of Thiamine, Riboflavin and Niacin

Thiamine content was determined using the method outlined by Okwu and Josiah[13] with some modifications. The extract (0.1 mL) was made up to 1 mL using distilled water. To this 0.1 mL of potassium dichromate was added and absorbance was measured at 360 nm.

For riboflavin estimation,[13] the extract (0.1 mL) was made up to 1 mL using distilled water. To this, 0.1 mL of 5% potassium permanganate and 0.1 mL of 30% hydrogen peroxide was added and allowed to stand over a hot water bath for 30 min. Then 0.02 mL of 40% sodium sulphate was added and absorbance was read at 510 nm.

For the estimation of niacin content,[13] the extract (0.1 mL) was made up to 1 mL using distilled water. To the solution, 0.05 mL of 0.1% potassium cyanide was added. The mixture was then acidified with 0.05 mL of 0.02 N sulphuric acid and the absorbance was measured at 470 nm.

2.7. Determination of Total Reducing Capability

Total Reducing capability was determined by the method of Oyaizu.[14] The aqueous extract was mixed with 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6). The dilute sample was then mixed with 5 mL of 1% potassium ferricyanide and the mixture was incubated at 50 °C for 20 min. 5 mL of 10% TCA was



Fig. 1. Photographs of different Medicinal rice cultivars used in this study (1) Joddubirunellu, (2) Ambimohar, (3) Karikalaveya, (4) Kagisali, (5) Kari gajivile, (6) Hakkalasali, (7) Njavara black, (8) Njavara yellow, (9) Chennellu, (10) Kullakar.

added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The supernatant (5 mL) was mixed with 5 mL of distilled water and 1 mL of ferric chloride (1%). The absorbance was measured at 700 nm.

2.8. Determination of Total Antioxidant Activity

Total antioxidant activity was determined by the method of Prieto et al.[15] The aqueous extract was mixed with 1 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated in a water bath at 95 °C for 90 min. After cooling to room temperature the absorbance was measured at 695 nm.

2.9. Statistical Analysis

Each experiment was performed in triplicate and the results were expressed as the mean values ± standard deviation. Duncan's

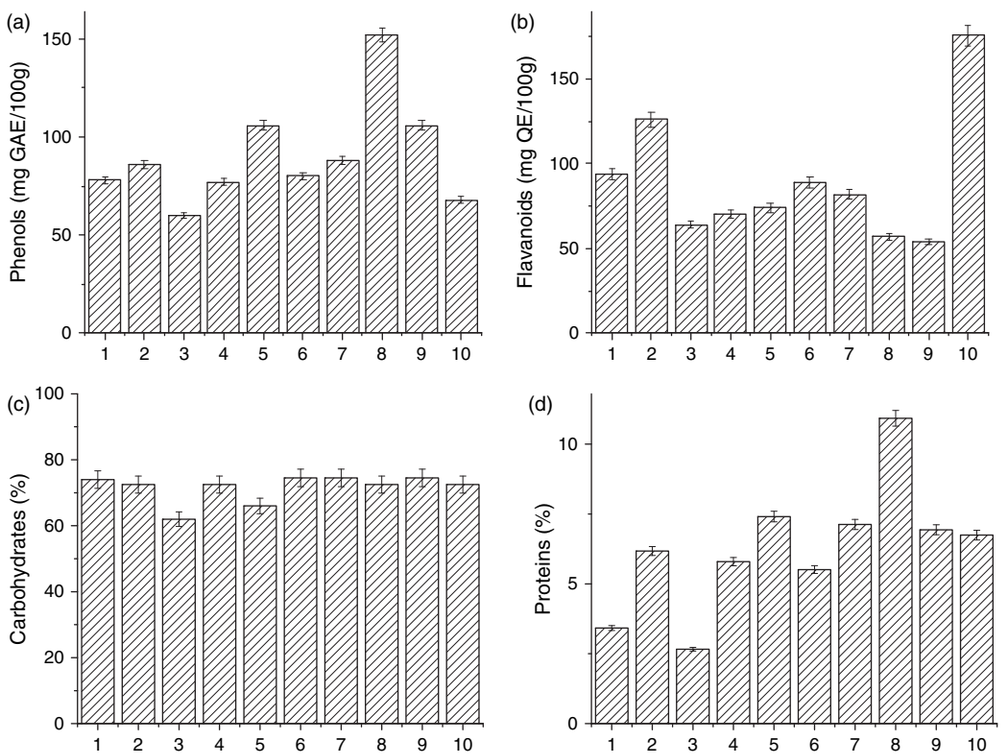


Fig. 2. Nutrient composition of 10 different medicinal rice varieties (a) Phenols, (b) Flavanoids, (c) Carbohydrates and (d) Proteins with the same order as shown in Table I.

multiple range test was conducted for comparison of means at $P < 0.05$.

3. RESULTS AND DISCUSSION

The composition of phenol, flavonoid, carbohydrate and protein is shown in Figure 2. The medicinal rice Njavara yellow showed the highest phenol (152 ± 3.80 μg/mL) and protein ($10.92 \pm 0.28\%$) content. Chennellu, Hakkalasali and Njavara black showed the highest carbohydrate ($74.5 \pm 2.65\%$) content. Kullakar of Tamil Nadu showed the highest flavonoid (176 ± 6.12 μg/mL) and thiamine (0.53 ± 0.01 mg/100 g) content. The vitamin content of the medicinal rice variety is shown in Figure 3. Riboflavin and Niacin content was high in Karikalaveya (0.08 ± 0.01 mg/100 g and 16 ± 0.58 mg/100 g respectively). The antioxidant property of the rice variety is shown in Figure 4. From the results of the estimation of reducing power and total antioxidant activity, Ambimohar and Njavara yellow showed the highest reducing power and Ambimohar had the highest total antioxidant activity.

The phytochemical studies of Njavara had been carried out by Rao et al.[16] They had compared the antioxidant and antiproliferative properties of Njavara with other non-medicinal rice varieties, and it was found that Njavara showed high levels of polyphenols, flavonoids, antioxidant activity and reducing power compared to the others. In the present study, we could find that all the six Karnataka varieties, Kullakar from Tamil Nadu and Chennellu from Kerala contain similar or higher levels of phytochemicals like phenols, flavonoids and alkaloids compared to Njavara varieties. Also the reducing power and total antioxidants of these cultivars are at par with Njavara varieties or higher. Reducing power has been used to evaluate the ability of natural antioxidants in the rice bran extracts to donate electrons.[17] Flavonoids have been widely used in cancer treatments, coronary heart diseases, gastrointestinal ulcers and rheumatic diseases.[18] The presence of phenolic compounds makes these rice varieties potential sources of antioxidants. The antioxidant activity of polyphenols is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.[19–22]

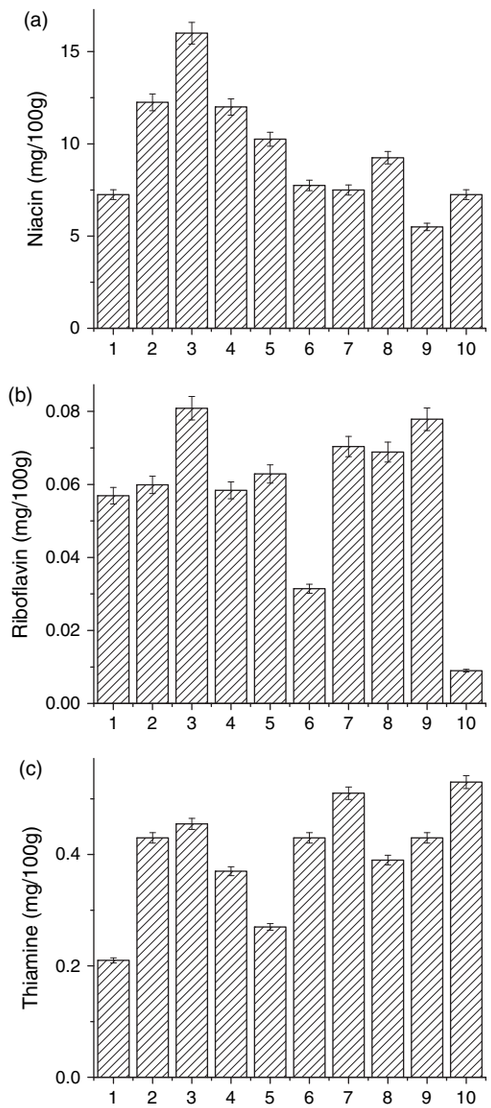


Fig. 3. Vitamin composition of 10 different medicinal rice varieties (a) Niacin, (b) Riboflavin and (c) Thiamine with the same order as shown in Table I.

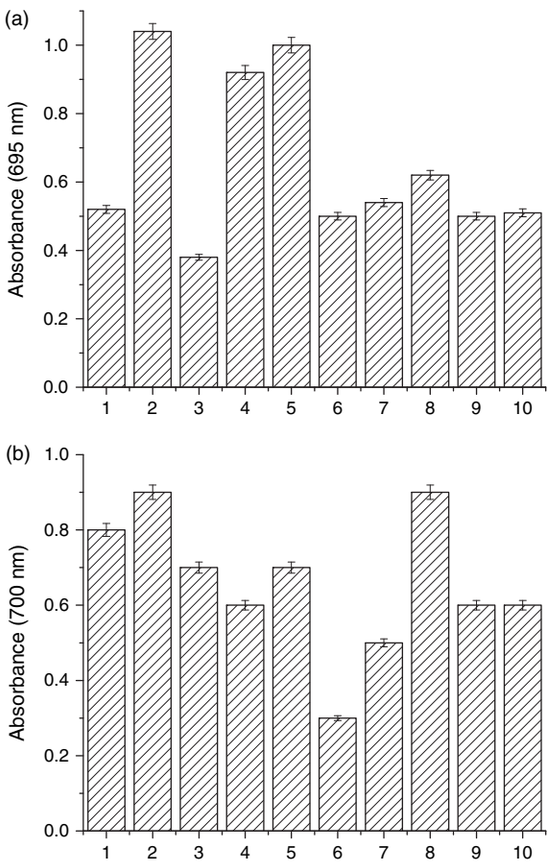


Fig. 4. Antioxidant properties of 10 different rice varieties (a) Total Antioxidant activity and (b) Reducing power with the same order as shown in Table I.

The nutritional composition of Njavara had been also compared with other non-medicinal varieties of rice by Deepa et al.[3] and Njavara showed a higher protein, carbohydrate, vitamins, minerals and fatty acid content. In the present study also, Njavara showed a higher carbohydrate, protein and riboflavin content. But the thiamine and niacin content were high in the other eight medicinal rice varieties compared to Njavara. Thus we have clear evidence that these medicinal rice varieties may also possess the remarkable properties of Njavara including its anticancerous and anti-inflammatory properties[16, 23–25] and high nutrient content.

4. CONCLUSION

Traditional knowledge on the medicinal properties of rice is available but proper scientific proof regarding the medicinal and nutritional value of most of the rice cultivars is absent. A thorough investigation of the physicochemical, molecular and genetic properties of the medicinal rice varieties is inevitable for their conservation as most of them are at the verge of extinction. Hence this study throws light on the phytochemical and nutritional aspects of the South Indian medicinal rice varieties, which may prove useful for further studies. In future these medicinal rice varieties could be used as a potential source for plant based pharmaceutical products. These rice varieties are of valuable genetic resources for the development of new and improved rice varieties with superior nutritional and medicinal values. Further studies aimed at confirming the anticancerous and antioxidative properties of medicinal rice cultivars are in progress.

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