INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print); 2222-5234 (Online)
Website: https://www.innspub.net
Email contact: info@innspub.net
Vol. 27, Issue: 3, p. 229-235, 2025

RESEARCH PAPER OPEN ACCESS

Assessment of antioxidant activity and GC-MS analysis of traditional Kavuni rice

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Key words: Black kavuni, Ethanolic extract, Anti-oxidant activity, Total antioxidant activity

DOI: https://dx.doi.org/10.12692/ijb/27.3.229-235 Published: September 26, 2025

ABSTRACT

The present study focused on evaluating the antioxidant potential and conducting GC–MS analysis of the traditional black rice variety, Kavuni. The total antioxidant activity of the rice samples was determined through standard assays, including hydroxyl radical scavenging, superoxide radical scavenging, nitric oxide radical scavenging, and total antioxidant activity tests. The black Kavuni rice exhibited the highest percentage of inhibition, ranging from 51% to 83%. Among the extracts tested, the ethanolic extract of Kavuni showed the greatest inhibitory effect across all antioxidant assays. GC–MS analysis revealed that phenolic acids and flavonoids were the predominant compounds in Kavuni grains, whereas sugars and fatty acids were more abundant in the white rice variety.

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INTRODUCTION

In India, traditional and pigmented rice varieties are known to possess a rich genetic diversity, encompassing valuable traits such as high nutritional content, medicinal properties, and distinctive aroma (Roy *et al.*, 2015), along with an unadulterated gene pool that serves as a source for important agronomic characteristics (Das and Das, 2014). The coloration in these rice varieties is primarily due to the accumulation of anthocyanins or proanthocyanidins in the outermost bran layer of the grain (Chaudhary, 2003).

Traditional rice varieties are abundant in minerals, bioactive compounds, and antioxidants (Vichapong *et al.*, 2014). Among them, black rice is a prominent pigmented variety traditionally cultivated in China, Thailand, and India (Rajeswari *et al.*, 2018). It belongs to the species *Oryza sativa* and is scientifically associated with the *Kala4* gene (an allele of *OsB2*), which regulates the biosynthesis of the anthocyanin pigment responsible for the dark coloration. Globally, more than 200 varieties of black rice have been reported (Zhang *et al.*, 2020), with major cultivation concentrated in Southeast Asian countries such as China, Thailand, and India. In India, black rice is mainly grown in the northeastern states—Manipur, Mizoram, Meghalaya, and Assam (Saha, 2016), whereas in Odisha it is locally known as Kalabati. In South India, particularly in the Karaikudi district of Tamil Nadu, it is cultivated on a smaller scale and popularly called Black Kavuni rice (Kumar and Murali, 2020).

Black Kavuni is a blackish-purple traditional rice variety of Tamil Nadu, often referred to as the "Emperor's Rice" due to its superior nutritional profile. The black pericarp of Kavuni rice is rich in phytochemicals, essential minerals such as iron, zinc, calcium, copper, sodium, potassium, and magnesium, and possesses remarkable therapeutic and nutritional properties that attract researchers and consumers alike. However, its cultivation remains limited among farmers because of its low-yielding agronomic traits (Kumar and Murali, 2020). To enhance its production and nutritional quality, twenty-three recombinant inbred lines (RILs) have been developed through biofortification efforts.

In the 21st century, black rice has gained recognition as a "superfood" owing to its high antioxidant content and vitamin E levels. The term superfood refers to foods with exceptional nutritional value. Historically, black rice originated in China and was regarded as a "lucky rice," believed to promote longevity due to its abundance of essential phytonutrients. Its deep black color is attributed to the presence of anthocyanins, powerful purple pigments concentrated in the bran layer. Anthocyanins, which make up about 26.3% of the pigments in black rice, are flavonoid compounds with strong antioxidant properties that protect the body from oxidative stress, aging, cancer, and other degenerative diseases.

MATERIALS AND METHODS

Paddy varieties such as black Kavuni (traditional variety), Rice was purchased from agriculture farm of Sundarakottai, TamilNadu, India. The DPPH assay was carried out based on the method suggested by Lim *et al.* (2007). The hydroxyl radical scavenging activity was measured by the procedure given by Halliwell *et al.* (1987). The superoxide anion scavenging activity and Nitric oxide scavenging activity were assessed by the method of Nishikimi *et al.* (1972) and Marocci *et al.* (1994). The total antioxidant activity was measured using ABTS assay (Gulcin *et al.*, 2007).

GC-MS analysis of the ethanolic seed extract of blackKavuni was performed using a Perkin Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer equipped with a Elite 5MS fused silica capillary column (30 × 0.25 mm ID. ×1 Mm df, composed of 5% Diphenyl/ 95% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 3 Ml was employed (split ratio of 10:1). Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10 °C/min to 200°C, then 5°C/min to 280°C ending with a 9 min isothermal at 280°C.

Mass spectra were taken at 70 eV, a scan interval of 0.5 seconds and fragments from 45 to 450 Da. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0. Interpretation on mass-spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library and the molecular weight and structure of the components of the test materials were ascertained (Gavamukulya, 2015).

RESULTS AND DISCUSSION

The DPPH assay revealed the total antioxidant activity of the rice samples, with mean inhibition values ranging from 16.5% to 83.2% at a concentration of 1000 µg/mL (Table 1).

Table 1. Antioxidant activity of black kavuni rice (DPPH)

Concentration (µg/mL)	DPPH (%)	IC50 (mg/mL)
100	16.5±1.02	0.85
250	34.7±0.7	
500	55.4±0.3	
1000	83.2±0.9	

These findings are consistent with earlier studies reporting high antioxidant potential in pigmented rice varieties. Reddy *et al.* (2015) observed DPPH scavenging activity between 89.28% and 92.67% in pigmented rice varieties from Manipur. Similarly, Meera *et al.* (2019) reported that black Kavuni rice exhibited a DPPH antioxidant activity of 95.48%. In comparison, Raghuvanshi *et al.* (2017) found that white rice showed only 20% DPPH scavenging activity, while red rice displayed 25% activity. Furthermore, Saikia *et al.* (2012) demonstrated that pigmented rice varieties exhibited antioxidant activity ranging from 94.19% to 96.43%, whereas non-pigmented varieties showed much lower values, between 30%and 35%.

Table 2. Hydroxyl radical scavenging activity of black kavuni rice

Concentration (µg/mL)	Inhibition (%)	IC50 (mg/mL)
100	15.7±0.02	0.67
250	29.2 ±0.8	
500	44.1±0.2	
1000	73.5±0.1	

The IC50 value of ethanol extract in rice samples ranged in 0.67 mg/ml (Table 2). Krishnanunni *et al.* (2015) reported that black Kavuni (IC50-0.67mg/mL) had ability to scavenge the hydroxyl radical. Compared to kuzhiyadichan rice, it had a reduced abilityto increase the concentration of Kavuni extract. And also showed that the phenolic compounds contain a positive correlation with antioxidant activity. Cho *et al.* (2012) reported that the ethanolic extract of black rice showed the strongest scavenging ability against hydroxyl radicals. Similarly in this study, the highest percent of hydroxyl radical scavenging activity was observed in Kavuni parent which might be a higher content of total anothocyanin.

Table 3. Superoxide radical scavenging activity of black kavuni rice

Concentration (µg/mL)	Inhibition (%)	IC50 (mg/mL)
100	7.23±0.42	2.4
250	11.5±0.7	
500	15.8 ±0.7	
1000	19.3±0.4	

The superoxide radical scavengingactivity percentage of the rice samples ranged from 7.23 to 19.3 percent (Table 3). The highest percent of inhibition was observed in Kavuni parent. Park *et al.* (2008) reported that the ethanolic extract of black rice contains the highest super scavenging activity than alpha-tocopheral due to the presence of anthocyanin. Pramai and Jiamyangyuen (2016) reported that, when compared to red and white rice, black rice contains the highest amount of total phenolic and flavanoids compounds. The L* (lightness) value of pericarp revealed a negative correlation with antioxidant activities. Similarly the present study shows that the Kavuni had the lowest L* (lightness) value due to the presence of black pericarp or anthocyanin. It was increased inhibition percent against superoxide radical.

Table 4. Nitric oxide radical scavenging activity of black kavuni rice

Concentration (µg/mL)	Inhibition (%)	IC50 (mg/mL)
100	3.125 ±0.5	3.5
250	6.25 ±0.8	
500	12.8 ±0.9	
1000	15.5±0.7	

Among parents, the Kavuni parent had the highest percent of inhibition and it was 15.5 percent, (Table 4). Reddy (2018) reported that the karuppu Kavuni had the ability to inhibit the nitric oxide radicals. A higher percentage of inhibition was observed (15.5%) in 1 mg/mL. Same way in this study the Kavuni rice had a higher percentage of inhibition against nitric oxide due to the presence of total phenolic content.

Table 5. ABTS radical scavenging activity of black kavuni rice

Concentration (µg/mL)	Inhibition (%)	IC50 (mg/mL)
100	4.8 ±0.7	2.1
250	9.2 ±0.6	
500	14.85±0.1	
1000	23.08±0.6	

The highest inhibition observed in ethanol extract of Kavuni rice was 15.5 percent. It had IC 50 value of 2.1 mg/mL (Table 5). Pramai and Jiamyangyuen (2016) reported that the Thailand black rice varieties contain higher total antioxidant activity than the red and white rice varieties. Among black rice varieties, the total antioxidant activity differed in the range between 5 to 12 mmol. Sompong *et al.* (2011). In similar way to the present study, higher total antioxidant activity was observed in Kavuni, may be the presence of phenolic compounds.

Table 6. Phytocomponents identified in karuppu kavuni [GC-MS]

Peak no.	R. Time	Area	Area%	Similarity	Name
1	7.891	1097514	0.63	95	Undecane
2	8.662	68256	0.04	89	2-Propyl-1-pentanol
3	13.293	157719	0.09	89	Cyclooctane,1,2-diethyl-
4	17.727	61389	0.03	89	Tridecane,5-methyl-
5	18.765	63949	0.04	91	E-14-Hexadecenal
6	19.946	539555	0.31	79	1,2,4,5-Cyclohexanetetrol,(1.alpha.,2.alpha.,4.alpha.,5.beta.)-
7	22.730	60754	0.03	88	Tridecane,5-methyl-
8	23.510	472876	0.27	95	DiethylPhthalate
9	23.694	65115	0.04	86	1-Tetradecene
10	23.879	1411592	0.80	98	Hexadecane
11	23.960	121994	0.07	83	Cyclopentane,1-hexyl-3-methyl-
12	24.507	136965	0.08	93	Benzophenone
13	27.432	419787	0.24	95	Tetradecanoicacid
14	27.578	340896	0.19	96	BenzylBenzoate
15	27.673	74883	0.04	85	Heptadecane,3-methyl-
16	28.150	42524	0.02	84	n-Nonadecanol-1
17	28.294	674316	0.38	96	Nonadecane
18	28.476	110755	0.06	84	Cyclotetradecane
19	29.437	279518	0.16	93	1,2-Benzenedicarboxylicacid,bis(2-methylpropyl)ester
20	31.130	156852	0.09	83	Undec-10-ynoicacid,dodecylester
21	31.334	216283	0.12	89	Phthalicacid,butylisohexylester
22	31.642	45643204	26.00	92	Octadecanoicacid
23	32.297	309818	0.18	94	Nonadecane
24	32.570	64153	0.04	80	Eicosylmethylether
25	34.118	69035	0.04	72	cis-13-Octadecenoicacid,methylester
26	34.835	41602626	23.69	95	10E,12Z-Octadecadienoicacid
27	34.956	78041962	44.45	93	cis-Vaccenicacid
28	41.720	158933	0.09	85	Oleoylchloride

29	42.700	93825	0.05	74	Isopropyllinoleate
30	46.963	486362	0.28	89	9-Octadecenoicacid,1,2,3-propanetriylester,(E,E,E)-
31	48.809	170184	0.10	93	Squalene
32	52.719	74722	0.04	73	Stigmast-5-en-3-ol,oleate
33	54.207	267948	0.15	71	(E)-5-((1S,5R,8aR)-5-Formyl-5,8a-dimethyl-2-methylenedecahydronaphthalen-1-yl)
34	55.335	404636	0.23	83	Campesterol
35	55.940	298003	0.17	74	Stigmasta-4,22-dien-3.beta.-ol
36	57.415	1318186	0.75	89	.gamma.-Sitosterol
		175577089	100.00		

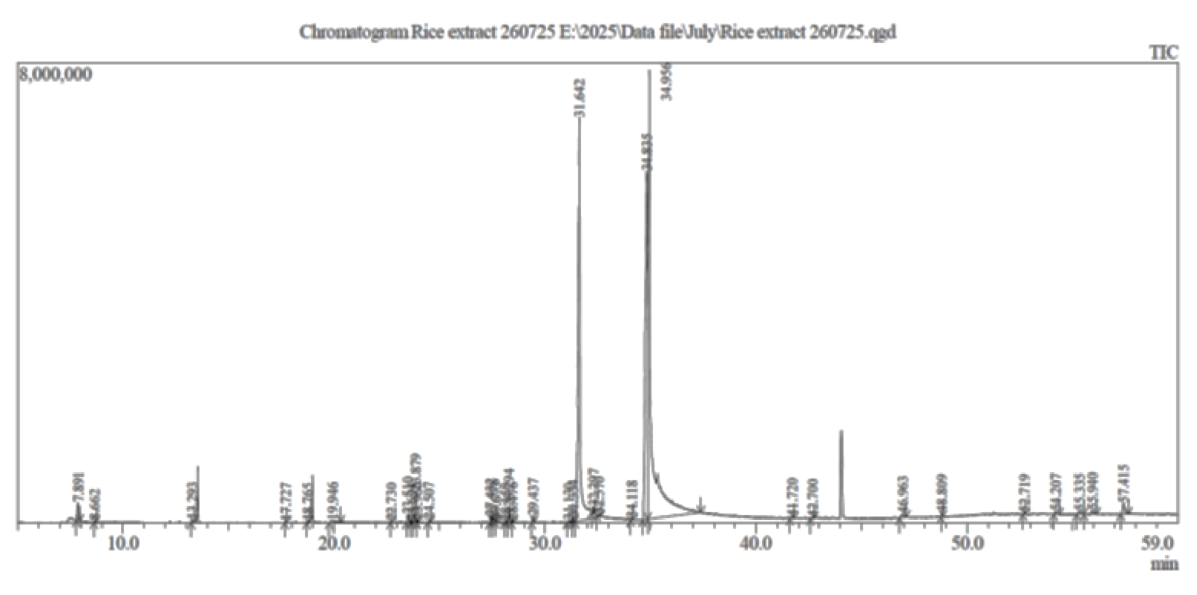

Fig. 1. Phytocomponents identified in Karuppu kavuni [GC-MS]

The GC-MS chromatogram of ethanol extract of KK shows 36 compounds detected was shown in Table 6 and Fig. 1. The ethanolic extract of KK showed the presence of major peaks and the components corresponding to the peaks were determined as follows. The results revealed that peak 1 – Undecaneat the retention time 7.891 ,peak 2 – 2-Propyl-1-pentanolat the retention time 8.662, peak 5-E-14-Hexadecenal at the retention time 18.765, peak 12- benzophenone with retention time 24.507, peak 13- tetra decanoic acid with retetntion time 27.432, peak 17- nonadecane with retention time 28.294, peak 22-octadecanoic acid with retention time 31.642, peak 31- squalene with retention time 48.809, peak 36- sitosterol with retention time 57.415 respectively. The identified compounds nonanal, decanol and flavones possess biomedical importance possess antimicrobial activity, having antioxidant properties and anti-inflammatory properties. The studies carried out by Zhang and groups confirm the anti-fungal activity of nonanal possesses antioxidant role and having pharmaceutical uses. The flavones have anti-inflammatory, anti-allergic, antioxidant, antimicrobial, and anti osteoporatic activity.

CONCLUSION

From the above study, it was completed that the recombinant inbred line 145-3 was similar to that of Kavuni parent in the context of antioxidant activity in DPPH assay, Hydroxyl radical scavenging activity, Superoxide radical scavenging activity, Nitric oxide radical scavenging activity and ABTS radical scavenging activity. Therefore the yielding character of Kavuni rice variety is without modification of therapeutic and nutrition properties. In view of the polished rice based foods and rice, consumption turned out to be the incidence of obesity and cardiovascular diseases. The kavuni rice owned high antioxidant properties than the white rice varieties. In this connection, the kavuni rice to be best functional food among diseased consumers.

ACKNOWLEDGEMENTS

We would like to express my sincere and heartfelt thanks to our beloved correspondent Dr. V. Dhivaharan, Department of Life Sciences, S.T.E.T. Women's College (Autonomous), Sundarakkottai, Mannargudi, for encouragement and providing adequate facilities in this research work successfully.

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