AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Phytochemical Composition and *In Vitro* Antidiabetic Efficacy of Selected Traditional Rice Varieties

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by sustained hyperglycemia due to impaired insulin secretion, insulin resistance, or both. Enzyme inhibition of α-amylase and α-glucosidase is a proven therapeutic strategy to attenuate postprandial glucose surges. Although synthetic inhibitors such as acarbose are widely used, their gastrointestinal side effects have stimulated interest in natural dietary alternatives. The present study investigates the phytochemical composition and in vitro antidiabetic activity of ethanolic extracts from five rice varieties: Mapillai Samba, Red Rice, Karuppu Kavuni, Karudan Samba, and Ponni Rice. Extracts were prepared using Soxhlet extraction and evaluated for α-amylase and α-glucosidase inhibition across concentrations ranging from 10 to 500 µg/mL, with acarbose serving as the reference inhibitor. Qualitative phytochemical screening revealed that all varieties contained alkaloids and flavonoids, while pigmented rice types exhibited higher diversity of secondary metabolites. In enzyme assays, Karuppu Kavuni showed the strongest inhibitory activity (91.40% for α-amylase and 91.10% for α-glucosidase at 500 µg/mL), closely comparable to acarbose (93.25% and 95.16%, respectively). Red Rice and Mapillai Samba demonstrated moderate activity, whereas Ponni Rice showed the least inhibition. Statistical analysis confirmed significant differences between rice varieties ($p < 0.05$). These findings suggest that pigmented rice varieties, particularly Karuppu Kavuni, may serve as functional foods or nutraceutical sources for managing postprandial hyperglycemia. Future in vivo validation and phytochemical characterization are warranted.

Keywords: Diabetes mellitus, α-amylase inhibition, α-glucosidase inhibition, pigmented rice, phytochemicals, Soxhlet extraction, functional foods

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Received 10 September 2025, Accepted 05 October 2025

Please cite this article as: Divya N *et al*., Phytochemical Composition and In Vitro Antidiabetic Efficacy of Selected Traditional Rice Varieties. American Journal of PharmTech Research 2025.

INTRODUCTION

Diabetes mellitus (DM) has emerged as one of the most critical global health challenges, with the World Health Organization reporting more than 422 million affected individuals worldwide (WHO, 2023)[1]. India alone accounts for approximately 77 million cases, making it the second largest contributor to the global diabetic population (IDF, 2021)[2]. Chronic hyperglycemia resulting from this disease contributes to complications including cardiovascular disease, nephropathy, retinopathy, neuropathy, and impaired wound healing.

Management strategies typically combine lifestyle modifications, pharmacotherapy, and insulin supplementation. However, the side effects and high cost of synthetic antidiabetic drugs highlight the need for safe, effective, and affordable plant-based alternatives. One proven therapeutic target is the regulation of postprandial hyperglycemia (PPHG) by inhibiting carbohydrate-digesting enzymes. α-Amylase, which catalyzes starch hydrolysis, and α-glucosidase, which cleaves disaccharides into absorbable glucose, are critical enzymes in carbohydrate metabolism. Inhibitors of these enzymes, such as acarbose and voglibose, effectively reduce glucose absorption but frequently induce gastrointestinal discomfort (Van de Laar et al., 2005)[3].

Plant-derived compounds, especially polyphenols, flavonoids, and anthocyanins, have been reported to exert α-amylase and α-glucosidase inhibitory effects (McDougall et al., 2005; Tadera et al., 2006)[4,5]. Rice (Oryza sativa L.), consumed by more than half the world's population, presents an excellent dietary source of such bioactive compounds. Pigmented varieties, in particular, are rich in anthocyanins, phenolic acids, and flavonoids, offering antioxidant, anti-inflammatory, and hypoglycemic properties (Sompong et al., 2011)[6].

This study was designed to investigate the phytochemical diversity and antidiabetic potential of ethanolic extracts from five traditional rice varieties: Mapillai Samba, Red Rice, Karuppu Kavuni, Karudan Samba, and Ponni Rice. The specific objectives were (i) to identify the phytochemical constituents of the extracts, (ii) to evaluate their α-amylase and α-glucosidase inhibitory activities in vitro, and (iii) to compare their efficacy against acarbose as the standard drug.

MATERIALS AND METHOD

Plant Material and Authentication

Grains of Mapillai Samba, Red Rice, Karuppu Kavuni, Karudan Samba, and Ponni Rice were procured from organic farms in Tamil Nadu, India, during the 2024 harvest season. The varieties were authenticated by a botanist, and voucher specimens were deposited in the herbarium of St. Joseph College, Trichy.

Sample Preparation

The grains were washed, shade-dried for 7 days at ambient temperature (27 ± 2 °C), and pulverized into fine powder using a Retsch ZM 200 laboratory mill. The powders were stored in airtight containers at 4 °C until extraction.

Extraction

One hundred grams of each powdered rice sample was extracted with 500 mL of ethanol (99.9%) using Soxhlet apparatus for 8 h at 65 °C. The extracts were concentrated with a rotary evaporator (Buchi R-300, Switzerland), dried in a vacuum desiccator, and stored at −20 °C in amber bottles until further use.

Phytochemical Screening

Standard phytochemical tests (Harborne, 1998[7]; Kokate, 2005) were conducted to detect alkaloids, flavonoids, phenols, tannins, quinones, saponins, terpenoids, and proteins in the ethanolic extracts.

α-Amylase Inhibition Assay

The α-amylase inhibitory activity was evaluated using Bernfeld's (1955)[9] DNSA method. Extract concentrations (10–500 µg/mL) were preincubated with 1 U/mL α-amylase for 10 min at 37 °C, followed by reaction with 1% soluble starch for 15 min. The reaction was stopped with DNSA reagent, boiled for 5 min, cooled, and absorbance was measured at 540 nm. Acarbose was used as the reference.

α-Glucosidase Inhibition Assay

The α-glucosidase inhibitory assay followed Kim et al. (2004)[8]. Extracts (10–500 µg/mL) were preincubated with 1 U/mL α-glucosidase in phosphate buffer for 10 min, followed by addition of pNPG substrate and incubation for 20 min at 37 °C. The reaction was terminated with sodium carbonate, and absorbance was recorded at 405 nm. Acarbose served as the standard.

Statistical Analysis

All assays were performed in triplicate. Results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA and Tukey's post hoc test with GraphPad Prism 9.0. Significance was set at $p < 0.05$.

RESULTS

Phytochemical Composition

Qualitative phytochemical analysis demonstrated that all rice varieties contained alkaloids and flavonoids, while pigmented varieties, especially Karuppu Kavuni, showed greater phytochemical diversity, including tannins, terpenoids, phenols, and saponins. Ponni Rice, a non-pigmented variety, displayed the narrowest phytochemical profile (Table 1).

Table 1. Phytochemical constituents of ethanolic extracts of various rice varieties.

S.No	Phytoconstituents	Mapillai samba	Red rice	Karupu kavuni	Karudan samba	Ponni rice
1	Alkaloids	+	+	+	+	+
2	Carbohydrates	-	+	+	+	-
3	Tannin	-	-	+	-	+
4	Terpenoids	-	-	+	+	-
5	Quinones	+	+	+	-	-
6	Total protein	-	+	+	-	+
7	Flavonoids	+	+	+	+	-
8	Phenols	+	-	+	-	+
9	Saponins	-	+	+	+	-

(+) indicates presence; (–) indicates absence.

α-Amylase Inhibition

All ethanolic extracts exhibited dose-dependent inhibition of α-amylase activity. At 500 µg/mL, Karuppu Kavuni recorded the highest inhibition (91.40%), comparable to acarbose (93.25%). Red Rice and Karudan Samba showed moderate inhibition (71.79% and 59.15%, respectively), whereas Ponni Rice exhibited the lowest inhibition (50.12%) (Table 2).

Table 2. α-Amylase inhibitory activity (%) of ethanolic extracts.

S.No	Sample concentration	Mapillai samba	Red rice	Karupu kavuni	Karudan samba	Ponni rice	Standard drug
1	10	19.443	19.087	27.60	16.32	12.12	30.21
2	50	24.839	33.190	52.70	25.49	20.67	42.02
3	100	26.805	45.879	73.20	36.21	29.49	59.45
4	250	31.022	58.320	80.10	49.31	38.59	71.69
5	500	38.349	71.786	91.40	59.15	50.12	93.25

α-Glucosidase Inhibition

Similarly, inhibition of α-glucosidase increased with concentration. At 500 µg/mL, Karuppu Kavuni again demonstrated the highest activity (91.10%), close to acarbose (95.16%). Mapillai Samba also showed substantial inhibition (82.87%), while Red Rice and Karudan Samba exhibited moderate effects. Ponni Rice had the weakest activity (53.14%) (Table 3).

Table 3. α-Glucosidase inhibitory activity (%) of ethanolic extracts.

S.No	Sample concentration	Mapillai samba	Red rice	Karupu kavuni	Karudan samba	Ponni rice	Standard drug
1	10	19.20	25.066	33.21	16.21	13.28	36.15
2	50	37.70	32.402	40.90	28.21	20.59	49.18
3	100	56.293	36.070	58.90	36.85	26.54	63.79
4	250	68.531	65.240	74.60	49.27	39.15	80.64
5	500	82.867	76.856	91.10	56.73	53.14	95.16

DISCUSSION

The study demonstrates that traditional pigmented rice varieties contain a broader spectrum of phytochemicals and display stronger antidiabetic potential compared to non-pigmented rice. The presence of flavonoids, phenols, and anthocyanins in Karuppu Kavuni and Red Rice likely accounts for their high enzyme inhibitory activity, consistent with prior reports (Huang et al., 2015; McDougall et al., 2005)[4,10].

The inhibitory selectivity of Mapillai Samba toward α-glucosidase highlights its potential for controlling disaccharide digestion, possibly minimizing the gastrointestinal side effects associated with broad α-amylase inhibition. Ponni Rice, despite its limited phytochemical profile, still demonstrated moderate activity, supporting the contribution of even minimal bioactive compounds to glucose regulation.

Overall, the findings reinforce the role of pigmented rice as functional foods with health benefits beyond basic nutrition. These results support dietary promotion of indigenous rice varieties for managing type 2 diabetes and emphasize the importance of biodiversity conservation.

CONCLUSION

This study establishes that pigmented rice varieties, especially Karuppu Kavuni, possess significant in vitro α-amylase and α-glucosidase inhibitory activity, with potency approaching that of the standard drug acarbose. The superior activity correlates with their diverse phytochemical profiles, particularly phenolic and flavonoid content. Mapillai Samba also demonstrated promising α-glucosidase inhibition, suggesting selective advantages for postprandial glucose regulation. Future research should focus on isolating and characterizing specific bioactive compounds, validating efficacy in vivo, and developing rice-based nutraceutical formulations for diabetes management. The findings further advocate the cultivation and consumption of traditional rice varieties, aligning health benefits with biodiversity preservation and sustainable agriculture.

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