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Evaluation of Anti-diabetic and Antioxidant Activities in Sivappukavuni Rice: A Strategic Approach for Diabetes Management

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Abstract: Traditional rice varieties are rich in minerals and vitamins like niacin, thiamine, iron, riboflavin, vitamin D, and calcium. Recent research highlights their higher fiber content, lower sugar levels, and antioxidative properties, making them beneficial for individuals with diabetes and effective in disease prevention. The current study focuses on the therapeutic qualities of Sivappukavuni traditional rice variety, in the context of diabetes treatment in comparison with a local cultivar CO 51. The antioxidant studies using the DPPH and FRAP assay proved the presence of the therapeutic potential of phytochemicals in the traditional rice variety. Further studies were carried out to confirm the antidiabetic properties in the Sivappukavuni which showed maximum alpha-amylase inhibition of 58.52% at the concentration of 250 µg/ml. According to the scale of a correlation coefficient, there exists a correlation between the antidiabetic property and antioxidant property showing a highly positive correlation. Thus, this ensures that antioxidant properties are responsible for the antidiabetic properties in the Sivappukavuni rice variety. The investigation of the Sivappukavuni traditional rice variety for the presence of such natural alpha-amylase inhibitors will help explore new sources and can be a booster for the reintroduction of such novel varieties in our daily diet based on proper scientific validation. Sivappukavuni rice could prove to be the source of such natural inhibitors and if adopted in a regular diet it can minimize the chances of people entering into the diabetic condition at an early stage of their life.

Keywords: Traditional rice variety, Antioxidant properties, Phytochemicals, Anti-diabetic properties

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Introduction

Rice is one of the most commercially important cereal crops and is a fundamental staple food in the global diet (Panda *et al.*, 2022). Rice is considered a “Queen among cereals” as it is the staple food for almost two-thirds of the population

in the world (Verma and Srivastav, 2017). Several secondary metabolites are present in rice cereal, including steroids, alkaloids, terpenoids, phenolic acids, and flavonoids (Fatchiyah *et al.*, 2023). Among the world nations, India and China are the

countries that primarily consume rice (Ren *et al.*, 2021). The productiveness and beneficial values of rice in these two countries have resulted in the introduction of thousands of rice varieties (Mbanjo *et al.*, 2020). Moreover, India has immense rice germplasms with various curative properties (Kowsalya *et al.*, 2022). They are grown in agro-meteorological environments and ecological conditions (Pandey *et al.*, 2012). Traditional rice varieties are known for their nutraceutical and beneficial properties as they possess an excellent source of minerals and vitamins such as thiamine, riboflavin, niacin, iron, calcium, and vitamin D with high fiber and low sugar content (Bhat and Riar, 2015). In recent years, more importance has been given to pigmented traditional rice varieties containing anthocyanin. Anthocyanin appears red when it is acidic and appears blue when it is alkaline. It contains several health benefits which include antiobesity, anti-diabetes, anticancer, and antiapoptosis (Laleh *et al.*, 2006; Lin *et al.*, 2017; Fatchiyah *et al.*, 2020; Sari *et al.*, 2021).

Diabetes Mellitus (DM) is a chronic disorder that prevails as a life threat to humankind because of high glucose levels, lifestyle components, and improper food habits that cause obesity (Sanada *et al.*, 2012; Taha *et al.*, 2022). WHO reports that by 2025 diabetic patient count may rise to 418 million and by 2030 it may reach 552 million (Sun *et al.*, 2022). Reports reveal that about 90% of diabetic patients are suffering from “non-insulin dependent Type 2 Diabetes Mellitus (T2DM)” (Ilea *et al.*, 2019). The enzyme α -amylase triggers the transformation of glucose from starch, whereas α -glucosidase controls the p-53 waving pathway and the partition of glucose from disaccharides (Yu *et al.*, 2012; Kalauni *et al.*, 2023). Thus, food containing these enzyme inhibitors effectively helps in the reduction of Type 2 Diabetes Mellitus (Kandasamy *et al.*, 2021). About 80% of people in the world use herbal medicines for the restoration of health (WHO, 2019).

Sivappukavuni, a traditional rice variety that originated in the Sivaganga district, is now widely

farmed across Tamil Nadu's delta areas. This type is well-known for its drought tolerance and disease resistance, and it thrives well in red soil conditions. It matures and is ready to harvest after around 140 days of growing. Among the other traditional rice types, Sivappukavuni was chosen for the study because it contains a high amount of antioxidants and has high amounts of iron, calcium, magnesium, potassium, zinc, and phosphorous (Sulochana *et al.*, 2016; Kalaivani *et al.*, 2020). The proanthocyanidins found in this rice variety protect against type 2 diabetes (Chen *et al.*, 2016). The focus of the current study is to prove with scientific validation the therapeutic potential of Sivappukavuni over the local cultivar CO 51 which is a high-yielding rice variety approved for cultivation across 14 states in India. In Tamil Nadu, it is suitable for all seasons (Kharif, Rabi, and Summer) (TNAU Portal). The acceptance of traditional rice varieties by a wide group of people based on scientific facts will pave the way to reducing the impacts and occurrence of diabetes. The fact that the traditional rice varieties which carry immense therapeutic properties and its negligence in recent times has kindled to pursue the current study.

Materials and Methods

Plant collection and preparation of the extract

The traditional rice variety Sivappukavuni (SK) and the modern rice cultivar CO-51 were obtained from Swami Dayananda Educational Trust Farm, Manjakudi, and Valanadu Sustainable Agriculture Producer Company Limited, Sirkazhi, in Mayiladuthurai District. The rice grains were cleaned, dehusked, and ground into a fine powder. Extracts were prepared using a 1:10 ratio with methanol and water as solvents in a Soxhlet apparatus, concentrated by air drying, and stored at 4°C for further analysis.

Qualitative analysis of phytochemicals (Secondary metabolites)

Preliminary analysis was done to identify the phytoconstituents such as alkaloids, carbohydrates, glycosides, saponins, proteins and amino

acids, phytosterols, and phenolic compounds present in the rice seed extracts prepared in methanol and aqueous using standard procedures (Harbonne, 1988)

Quantitative Analysis of Carbohydrates, Crude fiber, Total Phenols, Total Flavonoids

The quantitative analysis of carbohydrates, crude fiber, total phenols, and total flavonoids was estimated according to the methodology of Van Handel (1968), AOAC (1990), Singleton *et al.* (1999) and Zhishen *et al.* (1999).

Radical Scavenging activity -DPPH

The antioxidant activity was evaluated based on the hydrogen-donating or radical-scavenging ability using the stable DPPH radical (α , α -diphenyl- β -picrylhydrazyl), following the method of Kifle and Enyew (2020). Extracts of varying concentrations (20–100 μ l) were mixed with 1–2 ml of 0.1 mM DPPH, incubated in the dark for 30 min, and their absorbance at 517 nm was measured using a UV-VIS spectrophotometer, with DPPH inhibition (%) calculated as: DPPH inhibition (%) = $(Ac - Ao) / Ac \times 100$; where Ac is the absorbance of the control; Ao is the absorbance of the sample.

FRAP Assay

The ferric-reducing power of the rice extracts was assessed using the Benzie and Strain (1996) method. Rice extracts (20–100 μ l) were mixed with 1.3 ml of FRAP reagent, and incubated at 37°C for 30 min, and the absorbance was measured at 595 nm to determine the IC₅₀ value, representing the concentration of antioxidants required to reduce 50% of ferric radicals.

$$\text{FRAP inhibition (\%)} = (Ac - Ao) / Ac \times 100$$

Where Ac is the absorbance of the control; Ao is the absorbance of the sample

Alpha-amylase inhibition activity

The anti-diabetic activity of the test samples was evaluated using a modified method based on Jumeapaeng *et al.* (2013). Samples at varying concentrations (20–120 μ g/ml) were mixed with

methanol and alpha-amylase solution, incubated at 37°C for 10 min, and then combined with starch solution for a further 60 min incubation. The reaction was terminated with hydrochloric acid, followed by the addition of iodine solution, and the absorbance at 595 nm was measured, with a dark blue color indicating inhibition activity.

$$\text{Percentage of alpha-amylase enzyme inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Alpha-glucosidase inhibition assay

The alpha-glucosidase inhibition activity of the extracts was determined using a modified method of Kim *et al.* (2005). The reaction mixture, containing phosphate buffer, alpha-glucosidase, and varying concentrations of the extracts, was pre-incubated at 37°C for 15 min, followed by the addition of PNPG substrate and further incubation for 30 min. The reaction was stopped with sodium carbonate, and the yellow color intensity, measured at 405 nm, was used to calculate percentage inhibition, with acarbose as the standard and appropriate controls included.

$$\text{Percentage of alpha-glucosidase enzyme inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Statistical analysis

The experiments were conducted in triplicates. The data are presented as the mean $\pm$ SE and statistically analyzed using ANOVA in MS Excel.

Results and Discussion

This study analyzed the qualitative and quantitative phytochemical properties of seed extracts from Sivappukavuni (traditional rice variety) and CO-51 (local rice cultivar) from Tamil Nadu, India. Antioxidant activity was assessed using DPPH and FRAP assays, while the antidiabetic potential of Sivappukavuni was evaluated through alpha-amylase and alpha-glucosidase inhibition assays.

Qualitative and Quantitative Analysis of SK and CO-51

Phytochemicals can be defined as non-nutritious plant compounds that help either defense

Table 1: Phytochemical analysis of Sivappukavuni and CO-51 seed extract

S. No.	PHYTOCHEMICAL CONSTITUENTS	NAME OF THE RICE VARIETY			
		SIVAPPUKAVUNI		CO-51	
		Aqueous	Methanol	Aqueous	Methanol
1	Alkaloids	+	+	+	+
2	Carbohydrates	+	+	+	+
3	Glycosides	+	+	+	+
4	Saponins	+	-	+	-
5	Proteins and Amino acids	+	+	+	+
6	Phytosterols	-	+	-	+
7	Phenolic compounds and Tannins	+	+	+	+
8	Flavonoids	-	+	-	+
9	Gums and Mucilages	-	-	-	-
10	Volatile oil	-	-	-	-

+ indicates the presence of phytochemical constituent; - indicates the absence of phytochemical constituent

mechanisms or prophylactic purposes. They are mainly stored in the pericarp and bran of the rice seed. Thus, they help in preventing redox imbalance and advantageous cellular actions in the body (Amirtham and Radha, 2023). The secondary metabolites such as alkaloids, glycosides, saponins, phenolic compounds, flavonoids, phytosterols, and tannins were found to be present in both aqueous and methanolic extracts of both the rice cultivars whereas gums and mucilages and volatile oils are found to be absent in aqueous and methanolic extracts of SK and CO-51 rice varieties (Table 1). Anthocyanins were found to be present in the aqueous and methanol samples of SK and CO-51. A study on 10 Tamil Nadu traditional rice varieties reported the presence of phytochemicals such as alkaloids, glycosides, phytosterols, phenolic compounds, tannins, and flavonoids (Rajendran *et al.*, 2018). Ghasemzadeh *et al.* (2018) reported that the black, red, and brown rice bran of Malaysian rice varieties contain secondary metabolites, which is consistent with the findings of the present study.

Rice is an excellent source of complex carbohydrates. Carbohydrates will break down to glucose later to be used for vitality in performing activities and serve as fuel for the brain. People prefer slowly digestible starch-containing rice to escape type 2 diabetes (Chaudhari *et al.*, 2018). The total carbohydrates were higher in methanolic

extracts of SK (31.59 ± 0.04 mg/g) and lower in the aqueous extracts of CO-51 (6.5 ± 0.06 mg/g). The aqueous extract of SK (29.23 ± 0.02 mg/g) and the methanolic extracts of CO-51 (8.42 ± 0.06 mg/g) were found to be intermediate values as compared with the other extracts (Fig. 1). Carbohydrates are found to be higher in traditional rice variety compared with that of local rice cultivar CO-51.

Plant phenolics are known as principal antioxidants or free radical terminators that serve as important compounds of secondary metabolites (De Paulo *et al.*, 2021). Phenols belong to the group of natural antioxidants containing therapeutic functions. Some of the phenolic acids especially ferulic and p-coumaric acids are amply present in brown rice varieties, whereas the cyanidin-3-o- β -D glucoside and peonidin-3-o- β -D are present in the red and black rice varieties (Zhou *et al.*, 2004; Chen *et al.*, 2006; Yawadio *et al.*, 2007). The highest amount of total phenolics was present in the methanolic extracts of SK (36.1 ± 0.2 mg/g) followed by the aqueous extract of SK (25.7 ± 0.16 mg/g) and the lowest phenolics was found in the aqueous extract (15.03 ± 0.1 mg/g) of CO-51 (Fig. 2). A previous study (Nayeem *et al.*, 2021) on the phytochemical content and antioxidant activity of pigmented and non-pigmented Thai rice cultivars reported that the total phenolic content of methanolic extracts was higher than the aqueous, ethyl acetate and hexane.

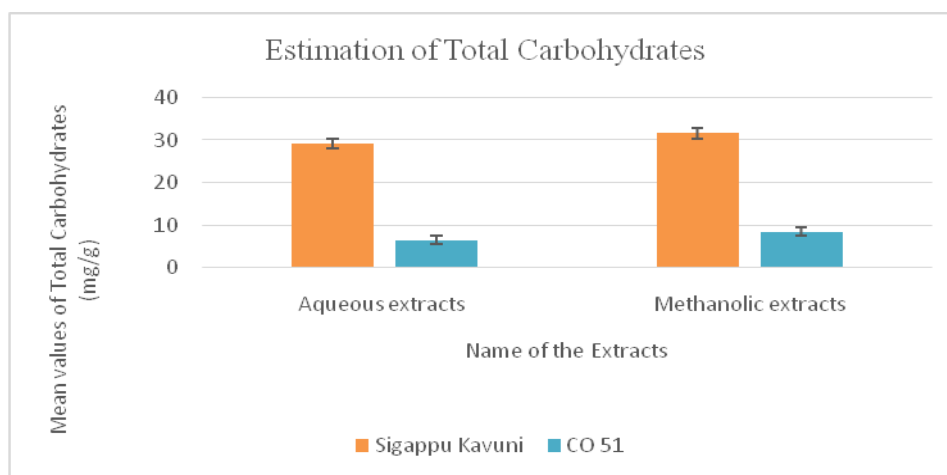


Fig. 1: Estimation of Total Carbohydrates of SK and CO-51.

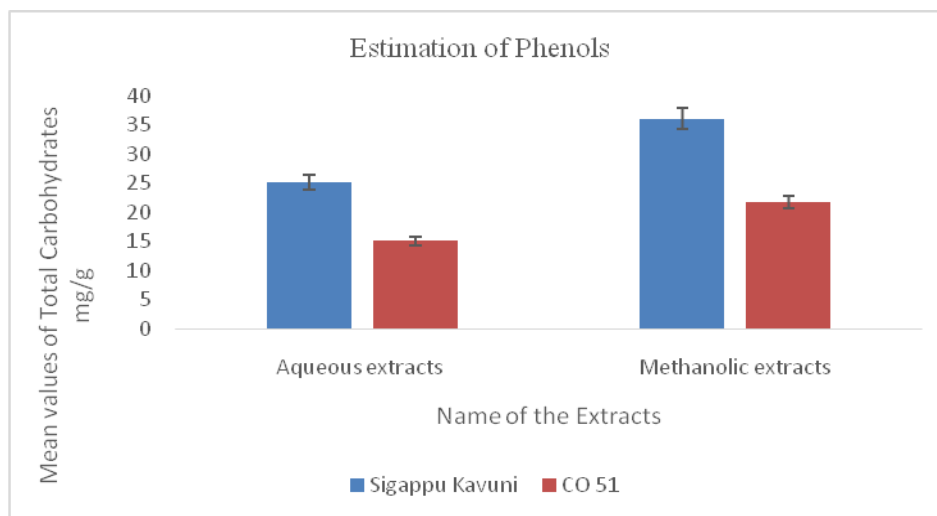


Fig. 2: Estimation of Total Phenols of SK and CO-51.

A study revealed that the polyphenol composition and antioxidant activity of the Japonica rice variety (a traditional rice variety in China) contains the highest number of phenols (Ma *et al.*, 2022). A recent study reported that ethanolic extracts of Kavuni rice contain high phenolics 29.91 ± 0.12 mg/g dry of gallic acid equivalent compared with the acetone extracts of the rice. It ensures that polar solvents are good in the extraction of phenolics (Sekar and Bhagavathy, 2023).

The phenolic compounds of rice are present in both soluble and insoluble forms as it is in other cereals (Adom and Liu, 2002). According to Furukawa *et al.* (2007) the accumulation of

anthocyanins contributes to the pigmentation of traditional rice varieties. The traditional rice variety SK contained higher flavonoids (71.4 ± 0.28 mg/g) when compared to the local rice variety (3.88 ± 0.03 mg/g) (Fig. 3). The result is similar to a study made on the black and red rice variety by Shen *et al.* (2009). Flavonoids exhibit antidiabetic activity by regulating carbohydrate digestion, insulin signaling, insulin secretion, glucose uptake, and adipose tissue deposition (Vinayagam and Xu, 2015). The traditional rice cultivar SK can prove to be a functional food source as it contains more flavonoids that aid in combating diabetes.

Radical scavenging activity -DPPH

The radical scavenging assay was based on

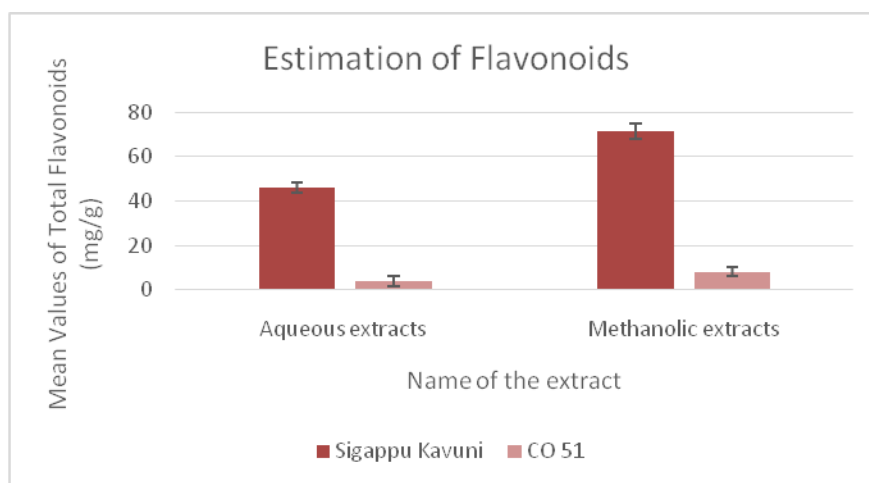


Fig. 3: Estimation of Total Flavonoids of SK and CO-51.

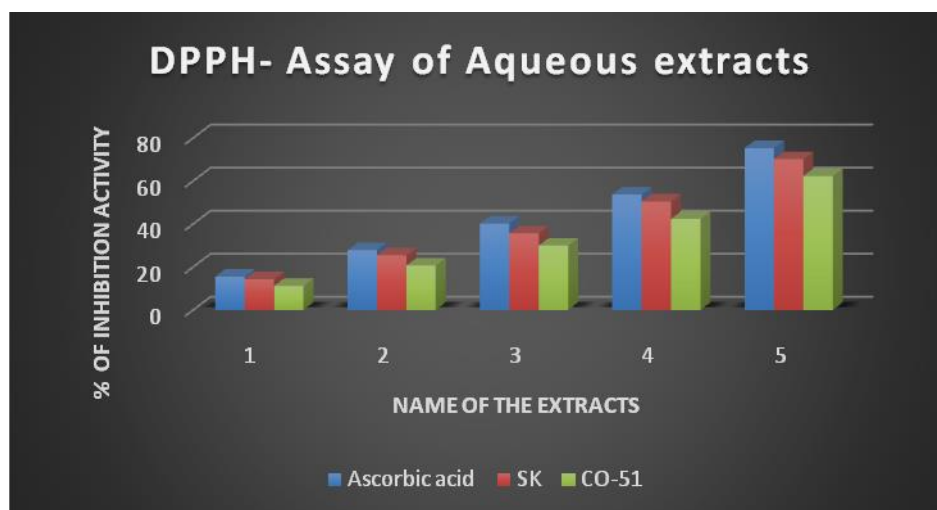


Fig. 4: DPPH assay of the Aqueous extracts of SK,CO-51 and Ascorbic acid showing the inhibition percentage of antioxidants.

shifting electrons from a donor to the corresponding radical. Several processes including free radical-scavenging, reducing capacity, metal-ion chelation, and inhibition of lipid peroxidation have been studied to use rice extracts as essential antioxidants (Sani *et al.*, 2012; Ghasemzadeh *et al.*, 2015). It is one of the simple methods to quantify the percentage inhibition of antioxidant activity. The molecule 1, 1-diphenyl-1,2-picryl hydrazine is characterized as a stable free radical by delocalization of the spare electron over the molecules as a whole. The maximum percentage of radical scavenging inhibition was observed in SK (72.01%) and CO-51 (52.02%) (Figs. 4, 5). Earlier studies have confirmed that the amount of

phenolics and flavonoids present in the rice variety is correlated with antioxidant activity (Zheng and Wang, 2001; Djeridane *et al.*, 2006; Ghasemzadeh *et al.*, 2015). The results indicated that the Traditional rice varieties with high radical scavenging ability could serve as good dietary source of antioxidants with beneficial effects leading to mitigate diabetic complications. The IC₅₀ (50% inhibitory concentration) values of aqueous and methanol extracts of SK, CO-51, and ascorbic acid are shown in Table 2.

Ferric Reducing Power Assay

Reducing the power of a biological substance is the capacity to transfer electrons, which may serve

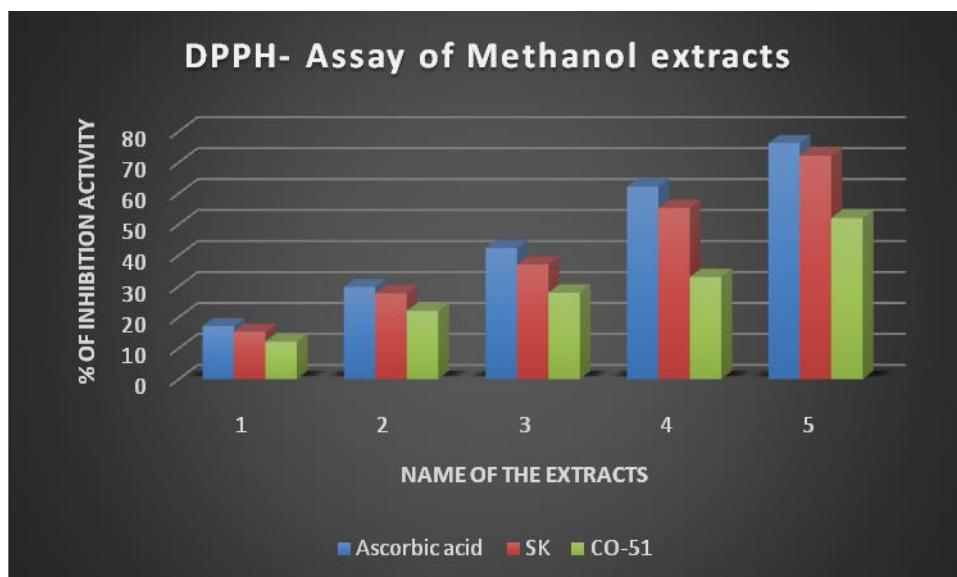


Fig. 5: DPPH assay of the Methanol extracts of SK,CO-51 and Ascorbic acid showing the inhibition percentage of the antioxidants.

Table 2: Fifty per cent inhibitory concentration (IC_{50}) of DPPH Assay

Name of the Sample	Aqueous Extracts (IC_{50}) Values	Methanol Extracts (IC_{50}) Values
Ascorbic acid (standard)	40.12	42.12
Sivappukavuni	23.01	24.52
CO-51	34.23	39.02

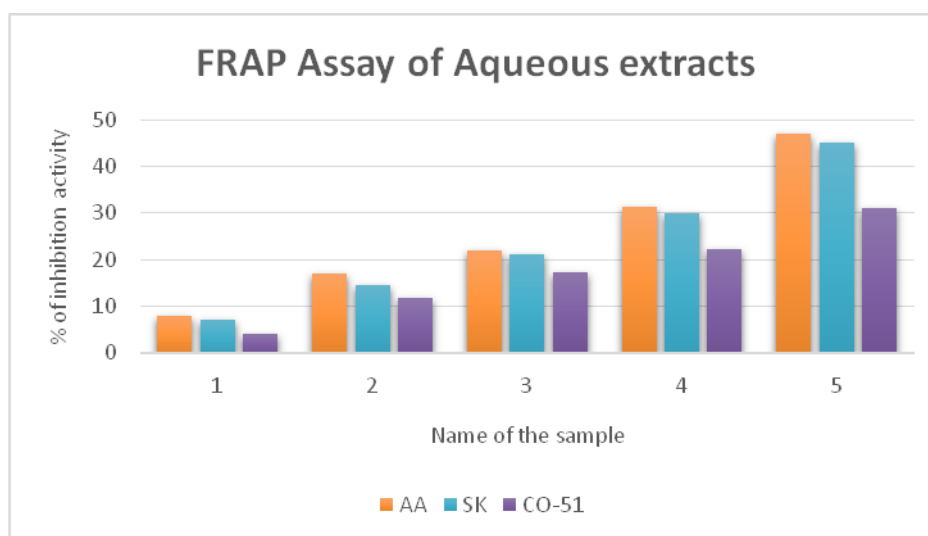


Fig. 6: FRAP assay of the Aqueous extracts of SK,CO-51 and Ascorbic acid showing the inhibition percentage of antioxidants.

as a sign of antioxidant potential (Balakrishnan *et al.*, 2019). In the current study, the results of the FRAP assay revealed that the observed FRAP values of the aqueous and methanol extract of SK and CO-51 fell in the range of 15.92 to 26.32%

(Figs. 6, 7). The CO-51 variety showed the lowest reducing power when compared to the SK variety. The result supports the fact that the antioxidants relatively depend on the reducing power of the bioactive compounds (Mir *et al.*, 2016). The

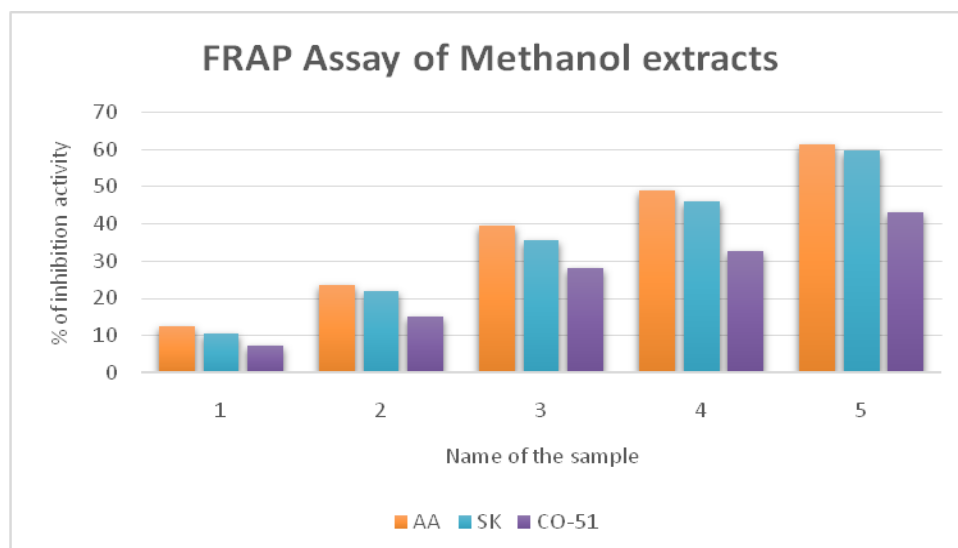


Fig. 7: FRAP assay of the Methanol extracts of SK, CO-51 and Ascorbic acid showing the inhibition percentage of antioxidants.

Table 3: Fifty per cent inhibitory concentration (IC₅₀) of FRAP Assay

Name of the Sample	Aqueous Extracts (IC ₅₀) Values	Methanol Extracts (IC ₅₀) Values
Ascorbic acid (standard)	22.21	26.32
Sivappukavuni	15.91	19.74
CO-51	20.14	21.98

correlations ($r = 0.824$, $p < 0.05$) found between phenolic compounds and antioxidant activity support the established relationship between these two characteristics. The IC₅₀ (50% inhibitory concentration) values of aqueous and methanol extracts of SK, CO-51, and Ascorbic acid are shown in Table 3.

Anti-Diabetic Assay

Alpha-amylase and alpha-glucosidase are crucial enzymes that are involved in the biochemical breakdown of starch into glucose, which is subsequently absorbed by the body. The inhibition of these enzymes plays a significant role in controlling excessive blood glucose levels, thereby regulating hyperglycemia, a condition that can lead to diabetes (Phoboo *et al.*, 2015). The inhibition of alpha-glucosidase enzyme is particularly effective in managing type 2 diabetes. This strategy prevents the rapid increase in blood glucose levels post-meal, providing a therapeutic approach to mitigate the adverse effects

associated with this chronic disease (Williams, 2006).

Inhibition of Alpha-Amylase Activity

The inhibition of alpha-amylase enzyme activity is a key strategy in the treatment of diabetes, proven to be highly effective in reducing postprandial hyperglycemia. This is achieved by targeting the hydrolysis of carbohydrates and the subsequent mobilization of glucose into the bloodstream at the intestinal interface (Agu *et al.*, 2019). Commercially, this can be accomplished by using chemical inhibitors such as acarbose, miglitol, and voglibose. However, the prolonged use of these chemical inhibitors can lead to severe gastrointestinal side effects, including abdominal pain, hepatitis, pancreatitis, and other cardiovascular diseases. Consequently, there is a necessity to identify and explore enzyme inhibitors from natural sources that have fewer side effects (Shahbaz *et al.*, 2023).

The experimental results obtained from

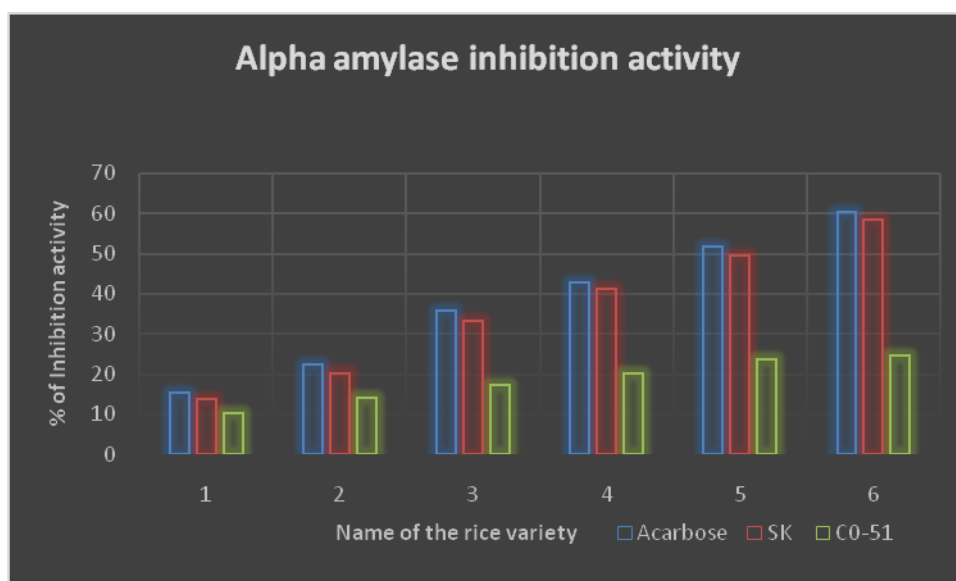


Fig. 8: Alpha amylase assay of the Methanol extracts of SK,CO-51, and Acarbose showing the inhibition percentage.

Sivappukavuni rice grain extracted with methanol as a solvent against the positive control acarbose resulted in maximum α -amylase inhibition of 58.52% at the concentration range of 250 $\mu\text{g/ml}$, while the CO-51 rice extract showed minimum α -amylase inhibition of 24.55% at the concentration of 200 $\mu\text{g/ml}$ (Fig. 8). The study indicates that the compounds in the SK rice variety are more soluble in methanol than in water. A study by Theiventhiran *et al.* (2022) characterized the antioxidant activity of recombinant inbred rice lines derived from traditional Kavuni and CO 51 varieties, using methanol as the solvent. The α -amylase inhibitory effect of Sivappukavuni extract was comparable to the clinically established drug at similar concentrations. For example, at a concentration of 200 $\mu\text{g/ml}$, the Sivappukavuni variety showed 49.43% inhibition while the acarbose showed 51.78% inhibitory activity. The results suggest that the activity of alpha-amylase enzyme was significantly suppressed by the methanolic extracts of kavuni rice grains which might be due to higher amounts of phytochemical constituents (phenols, flavonoids, and anthocyanins) present in them.

Ali Sagar (2013) reported that in Type-2

diabetic patients, the enhanced activity of α -amylase enzyme may be due to non-covalent interactions that occur between polyphenolic compounds and proteins. Also, the phenolic compounds can form hydrogen bonds with polar groups of enzymes which could result in managing the postprandial hyperglycemia condition. The results confirmed that a higher number of phytochemical constituents extracted from Sivappukavuni has shown good inhibition against alpha-amylase enzyme and can be consumed in managing Type 2 diabetic conditions.

Inhibition of Alpha-Glucosidase Activity

Inhibiting α -glucosidase enzyme activity is a therapeutic strategy to prevent Type 2 diabetes by delaying glucose absorption into the bloodstream (Dirir *et al.*, 2022). The maximum α -glucosidase enzyme activity was observed in extracts from Sivappukavuni rice, reaching 75.09% at a concentration of 300 mg/ml, whereas the minimum activity was noted in CO-51 rice extracts, with 13.02% at a concentration of 50 mg/ml (Fig. 9). These results indicated that methanolic extracts of Sivappukavuni rice grains retain higher levels of intact anthocyanins in the aleurone layer of the rice. This finding is

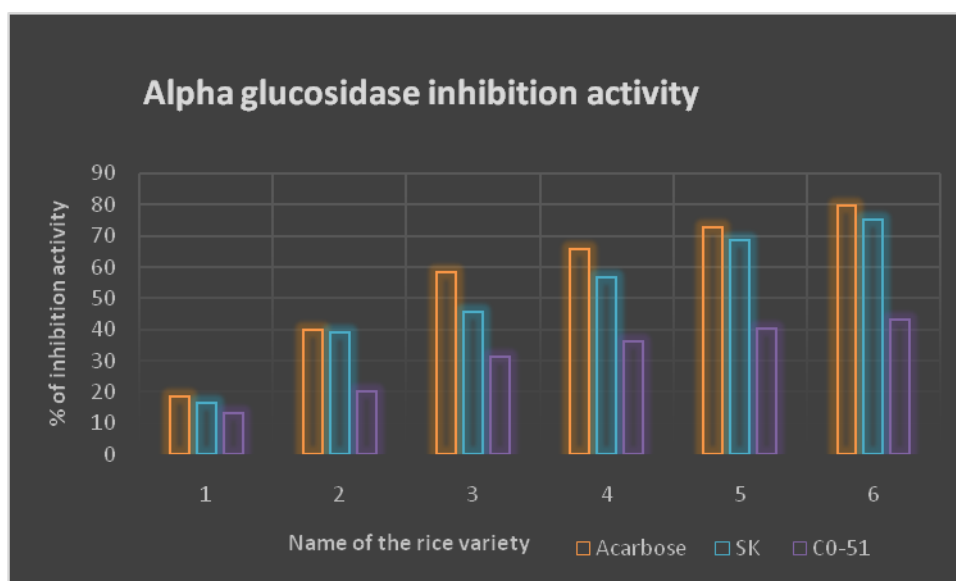


Fig. 9: Alpha-glucosidase inhibition assay of the Methanol extracts of SK, CO-51, and Acarbose showing the inhibition percentage of the enzyme.

consistent with the study by Barik *et al.* (2020), who demonstrated that anthocyanins extracted from black currant berries exhibited superior α -glucosidase inhibitory activity compared to phenolic compounds. The findings of this study suggest that the anti-hyperglycemic effect of Sivappukavuni grains is primarily driven by the synergistic interaction between phenolic compounds and anthocyanins. This conclusion is further supported by *in silico* studies, which reveal a complex binding pattern between the phytochemicals present in the grain extract and the digestive enzymes under investigation (Kato-Schwartz *et al.*, 2020). Consequently, our results on the inhibition of carbohydrate-hydrolyzing enzymes support that consumption of Kavuni rice grains could effectively regulate postprandial glucose levels in diabetic patients with minimal side effects (Valarmathi *et al.*, 2015)

Relationship Between Antioxidant Inhibition Activity and Anti-diabetic Activity

The correlation analysis was done between the antioxidant assay and antidiabetic assay of methanol extracts of SK and CO-51 rice variety. According to the scale of the correlation coefficient, the results obtained showed that there exists a highly positive correlation ($r = 0.969$,

$p < 0.05$) between antioxidant and anti-diabetic values of the SK traditional rice variety, indicating that the higher antioxidant value resulted in higher anti-diabetic value. This suggests that the antioxidant properties in the SK rice variety were highly responsible for their anti-diabetic capacity.

Conclusion

Traditional rice cultivars are highly known for their nutrient and anti-diabetic properties but scientific validation of the medicinal and nutritional value is lacking. Thus, the present study aimed to prove that the Sivappukavuni a well-known traditional rice variety contains a high amount of phenols, flavonoids, antioxidants, and anti-diabetic properties with that of the local rice variety. Thus, this study sheds light on the phytochemical and anti-diabetic aspects of the traditional rice variety Sivappukavuni and emphasizes the fact that the antioxidant properties positively correlate with anti-diabetic activity. In future, these medicinal rice varieties can serve as functional food. The prevalence of diabetes has been steadily increasing among people in the past few decades, especially in Tamil Nadu at about 10.42% as seen in a study by the Indian Council of Medical Research. Diabetes may

become the World's seventh-largest killer by 2030 (WHO, 2020). The presence of alpha-amylase inhibitors accompanied by free radical scavenging properties in Sivappukavuni may help to combat type II diabetes effectively through dietary supplements. Since Sivappukavuni is rich in antioxidants and antidiabetic properties it can be administered for normal people as well as diabetic patients.

Ethical Statement

No animal or microbes have been used or sacrificed for this study, hence Ethical Approval not required.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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