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Revealing The Nutritional, Antioxidant and Antidiabetic Potentials of Kattuyanam- An Indigenous Rice Variety Of Tamil Nadu

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Abstract
Diabetes is a chronic metabolic disorder which occurs when the body fails to utilize glucose properly. Even though a number of factors are associated with the cause of diabetes, higher consumption of polished white rice is related with an increased risk of it in recent years. On the other hand, the pigmented rice varieties are getting research of interest since are enriched with a number of phytoconstituents and antioxidants. Kattuyanam, one such pigmented rice variety of Tamil Nadu is very well known for its antidiabetic property and low glycemic index. But scientific knowledge about the rice variety is not reported till date. The present study was aimed at revealing the nutritional property of Kattuyanam rice along with its antidiabetic efficiency. To our best knowledge, the present work is the first description on the nutritional and antidiabetic potential of Kattuyanam rice. The nutritional profiling of the Kattuyanam rice showed that it contained lowered sugar levels and fat content, increased dietary fiber and protein content. The scavenging assay of the extracts of Kattuyanam rice exhibited good antioxidant properties with significantly higher inhibition rate. Since the antidiabetic property residing in the Kattuyanam rice is not yet revealed scientifically, this study aims in evaluating the same through *in vitro* methods. The glucose diffusion inhibition, significant glucose adsorption capacity and enhanced uptake of glucose by the extracts of Kattuyanam rice proved its antidiabetic properties. The results of the present study will pave a way for developing a diet strategy for managing Diabetes mellitus.

Keywords- Diabetes, Coloured rice, Kattuyanam, Antioxidant, Anti-diabetic

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia. The incidence of diabetes is rising at an alarming condition globally with a record that around 463 million people were affected with the disorder in 2019 and it is estimated that by 2045 that it will be increased to 700 million [1]. Apart from genetic basis and lifestyle pattern, diet plays a critical role in causing this metabolic disorder. Higher consumption of rice is also attributed towards the development of diabetes in particular Type II diabetes [2]. Rice is the staple food crop consumed by over half of the world's population. With the advancement of the grain processing technology, the removal of the outer bran of the intact rice grains (i.e., brown rice) is done to make it more attractive and dazzling. The bran which contains plenty of nutrients are completely removed from the whole grain, thus making it full of starch and devoid of proteins, vitamins, minerals, functional nutrients and fibres [3]. A study conducted by the researchers of the Harvard University communiqued that consuming one cup of polished white rice everyday will definitely lead to Type 2 Diabetes irrespective of the nationality or family history of the disease. Regular consumption of White rice as the primary food increases the susceptibility of getting diabetes by elevating the Postprandial Blood Glucose levels [4]. This predilection of White rice to raise the blood glucose levels after consumption makes it a food with High Glycemic index (GI). Usually the low glycemic food release glucose slowly and steadily thereby keeping the blood sugar levels more stable which is a crucial part of diabetes management. On the other hand, foods with high glycemic index gets easily absorbed which simultaneously increases the postprandial blood glucose level and insulin demand leading to diabetes[5]. Polished white rice falls under high glycemic foods category. Unlike white rice, pigmented rice varieties slowly releases sugars which in turn helps to stabilize blood sugar sustainably. This trait makes it a better option for people who are suffering from Diabetes Mellitus [6].

India has a rich biodiversity of traditional pigmented rice varieties with medicinal properties. This includes red, brown and black rice which are named based on the colour of the paddy. These rice varieties are highly nutritious than the polished white rice and are still cultivated and consumed in many parts of the country [7]. Kattuyanam is one such indigenous rice variety commonly cultivated in the Southern state of the India called Tamil Nadu. It is a pigmented rice variety usually grown in the Coastal areas of Tamil Nadu which can sustain higher water salinity. It was mainly given to children to help strengthen the bone marrow growth, thus improving immunity; it also improves vision and prevents skin diseases [8]. In a view of finding an alternative to the polished white rice, a medicinal rice variety of Tamil Nadu

named Kattuyanam was found to possess high nutritional content along with antidiabetic properties. The present study was undertaken to report the nutritional, antioxidant and therapeutic potential of Kattuyanam rice.

The nutritional profiling of the rice varieties is the major criteria for planning the diet requirements. The natural antioxidant defense mechanism protects the human body from oxidative damage. Because of the inadequate presence of natural antioxidants, the nutritional consumption of it from plants as the primary source is highly recommended nowadays. These antioxidants function as free radical scavengers, reduction agents and plays a significant role in delaying the progression of many chronic illnesses including cancer and stroke and prevents lipid oxidative rancidity in foods. With respect to anti-diabetic potential, Glucose adsorption, Glucose diffusion inhibition and glucose uptake are also potent indicators for analysing and identifying the anti-diabetic properties of extracts [9].

2. Materials and Methods

2.1 Material collection and sample preparation

Grains of Kattuyanam were procured from the farmers of Thanjavur district of Tamil Nadu. The rice varieties was tested at the Rice research station in Ambasamudram.

2.2 Physicochemical properties

To evaluate gel consistency, Kattuyanam rice powder was mixed with ethanol containing thymol blue and potassium hydroxide. The mixture was heated in a boiling water bath for 8 minutes, cooled in ice water, and placed horizontally on a ruled paper to measure the gel length after 30-60 minutes. For aroma detection, 200 mg of rice was treated with potassium hydroxide and left undisturbed for 15 minutes. The aroma was detected by smelling the open tube after incubation [10].

2.3 Proximate analysis

The proximate composition of Kattuyanam rice was analysed using standard methods. Moisture content was determined by the oven-dry method, measuring the weight loss of the sample at 105°C. Total nitrogen was assessed using micro-Kjeldahl method, and crude protein was calculated as Total Nx5.95. Dietary fibre, total ash and crude lipid content were also estimated through standard procedures. Crude fat content was determined using Soxhlet extraction with petroleum ether, with fat content calculated as a percentage based on the weight of fat in the sample [11].

2.4 Estimation of total soluble sugars and amylose content

To estimate total soluble sugars, the rice grain was finely powdered, and the phenol sulphuric acid method was employed for analysis. Starch content was determined using the anthrone method with glucose as a standard. Amylose content was estimated from defatted rice flour, with defatting done using 85% methanol and potato amylose as the standard [12].

2.5 Determination of food energy

The gross food energy was calculated using the formula: Gross energy (kCal/g) = (CPx4) + (Fx9) + (CHOx4) where CP is crude protein, F is fat, and CHO is total carbohydrate content.

2.6 Phytochemicals, antioxidant and antidiabetic activities of Kattuyanam

2.6.1 Sample extraction

Shade-dried Kattuyanam grains were powdered using a mortar and pestle. About 10 g of the powdered sample was mixed with 100 mL of 50% methanol and 50% ethanol individually and extracted in the dark for 48hours. The extracts were then filtered using Whatman No. 1 paper, and the filtrates were concentrated in a rotary evaporator under reduced pressure at temperatures below 45°C. The concentrated extracts were stored in refrigerated conditions for further analysis.

2.6.2 Estimation of Anthocyanin content

To determine the total monomeric anthocyanin content of Kattuyanam rice, about 0.5 mL of the rice extracts were mixed with 3.5 mL of 0.025 M potassium chloride buffer (pH 1) and vortexed. After 15 minutes of incubation, the absorbance was measured at 515 nm and 700 nm. The process was repeated using 0.025 M sodium acetate buffer (pH 4.5), and the absorbance again measured. The total anthocyanin content was calculated using the formula involving absorbance values, molecular weight, dilution factor, molar absorptivity and buffer concentration [13].

2.6.3 Estimation of total phenols

For total phenol estimation, 0.5 ML of rice extracts were mixed with water, Na_2CO_3 and Folin-Ciocalteau reagent, and the absorbance was measured at 725 nm. The results were expressed as gallic acid equivalents (GAE) [13].

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2.6.4 Estimation of total flavonoids

The total flavonoid content was determined by colorimetric method. Standard solutions were mixed with sodium nitrate, aluminium chloride and NaOH, then diluted to 10 mL with water. The absorbance was read at 510 nm, and a calibration curve was plotted to determine flavonoid concentrations in the rice extracts. [13]

2.6.5 Evaluation of antioxidant activity

The antioxidant activity of the extracts of Kattuyanam rice was measured in terms of DPPH radical scavenging, Ferric reducing antioxidant power, Hydroxyl radical scavenging, Superoxide anion scavenging, and Nitric oxide scavenging activity [14]. The brief procedure employed for measuring the antioxidant activity is mentioned below:

2.6.5.1 Determination of DPPH radical scavenging activity

The extracts of Kattuyanam rice were tested for its antioxidant activity using the DPPH method. DPPH, a purple radical, turns yellow upon reduction by antioxidants. A 100 µm DPPH solution was mixed with different concentrations of extracts (20-100µg/mL) and incubated at 27°C for 20 minutes. Absorbance was read at 517 nm, and the scavenging percentage was calculated using the formula,

DPPH Scavenged (%) = $[(A_0 – A_1)/ A_0] \times 100$

Where A_0 is the absorbance of the control, A_1 is that of the sample. Gallic acid was used as positive control.

2.6.5.2 Estimation of Ferric reducing antioxidant power

The ferric reducing power of Kattuyanam rice extracts were determined using the potassium ferricyanide-ferric chloride method. Extracts were incubated with phosphate buffer and potassium ferricyanide, followed by trichloroacetic acid and centrifugation. The upper layer was mixed with $FeCl_3$, and absorbance was read at 700 nm. Increased absorbance indicated higher reducing power. Quercetin were used as positive control.

2.6.5.3 Hydroxyl radical scavenging activity

For hydroxyl radical scavenging, rice extracts at different concentrations were mixed with Fe-EDTA, EDTA, DMSO and ascorbic acid, and incubated at 85˚C. After incubation, TCA and Nash reagent were added, and the absorbance was measured at 412 nm. The Hydroxyl radical scavenging ability was calculated using the formula:

% HRSA = $\{(A_0-A_1)/A_0\} \times 100$

With A_0 as the absorbance of the control and A_1 as the absorbance of the sample. Catechin was used as the positive control.

2.6.5.4 Nitric oxide radical scavenging activity

The nitric oxide scavenging ability of rice extracts were determined using the Griess reagent method. Different concentrations of the extracts were incubated with sodium nitroprusside at 37˚ C for 1 hour. The Griess reagent, prepared from sulphanilamide and naphthylethylenediamine dihydrochloride in phosphoric acid, was added after incubation. The absorbance of the resulting chromophore was measured at 570 nm. The scavenging percentage was calculated using the formula:

% of nitric oxide scavenged = $\{(A_0-A_1)/A_0\} \times 100$

Where A_0 is the absorbance of the control and A_1 is the sample absorbance. Curcumin served as the positive control.

2.6.5.5 Superoxide anion scavenging activity

To measure superoxide anion scavenging ability, the reaction involved NiroBlue Tetrazolium (NBT), Nicotinamide adenine dinucleotide (NADH), and phenazine methosulfate (PMS) in phosphate buffer. Various concentrations of the rice extracts were mixed and incubated at 25°C for 5 minutes. The absorbance of the reduced NBT, forming purple formazan, was measured at 562 nm. Ascorbic acid was used as a positive control, and the scavenging percentage was calculated as:

% of anion scavenging activity = $\{1- A_{Sample}/A_{Blank}\} \times 100$

Where A_{Sample} is the absorbance of the test sample or standard and A_{Blank} is the absorbance of the blank.

2.6.5.6 Total antioxidant activity

For total antioxidant capacity, different concentrations of the rice extracts were incubated with a reaction mixture containing sulphuric acid, sodium phosphate, and ammonium molybdate at 95°C for 10 minutes. The absorbance was measured at 695 nm, and Catechin was used as the positive control. Increased absorbance of the reaction mixture indicated increased total antioxidant activity.

2.6.6 In vitro antidiabetic activity analysis

2.6.6.1 Glucose uptake Assay

L-6 cells were cultured in DMEM (4.5 g/L glucose) with 10% fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 µg/mL streptomycin. Cells were incubated at 37˚C with 5% CO2, and sub-confluent cultures were maintained by

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trypsinization. The cells were then seeded in 6-well plates and incubated for 48 hours. After reaching semi-confluency, the medium was replaced with serum-free DMEM (0.2% BSA) and incubated for 18 hours. Cells were washed with KRP buffer and treated with insulin, metformin or rice extract (ethanol and methanol) followed by the addition of 1M glucose. After 30 minutes, the supernatant was collected for glucose estimation and the cells were lysed for further analysis. Glucose uptake was measured as the difference in glucose concentration before and after treatment using the GOD-POD method. Eight groups (n=5 wells each) were tested and was depicted in Table 1

Table 1 Experimental group for Glucose Uptake Assay

Group	Incubation Medium
1	Control group (900 µl of KRP buffer and 100 µl of glucose)
2	800 µl of KRP buffer, 100 µl of Insulin and 100 µl of glucose
3	800 µl of KRP buffer, 100 µl of metformin and 100 µl of glucose
4	700 µl of KRP buffer, 100 µl of Insulin, 100 µl of metformin and 100 µl of glucose
5	800 µl of KRP buffer, 100 µl of rice extract (ethanol) and 100 µl of glucose
6	800 µl of KRP buffer, 100 µl of rice extract (methanol) and 100 µl of glucose
7	700 µl of KRP buffer, 100 µl of Insulin, 100 µl of rice extract (ethanol) and 100 µl of glucose
8	700 µl of KRP buffer, 100 µl of Insulin, 100 µl of rice extract (methanol) and 100 µl of glucose

2.6.6.2 Glucose adsorption assay

To evaluate the Glucose adsorption capacity of the rice extracts, 1g of each extract was mixed with five different glucose concentrations (5, 10, 15, 20 and 30mM) in 100 mL of solution. The mixture was stirred gently and incubated in a shaker water bath for 6 hours at 37°C. After incubation, the mixture was centrifuged at 4800 rpm for 20 minutes. The glucose content in the supernatant was determined using a glucose oxidase peroxidase diagnostic kit. The amount of glucose bound was calculated using the formula

$$\text{Glucose bound} = \frac{G1-G6}{\text{volume of sample}} \times \text{volume of sample}$$

Where G1 is the initial glucose concentration and G6 is the glucose concentration after incubation [15].

2.6.6.3 Glucose diffusion inhibition assay

The Glucose diffusion inhibition assay was performed using dialysis tubing. A dialysis tube containing 2 mL of each rice extract (in 0.15 M NaCl) and 22 mM glucose was sealed and placed into a conical flask containing 45 mL of 0.15 M NaCl. The conical flask was placed into an orbital shaking incubator at 37°C at 100 rotations per minute. Aliquot (10 µl) of the external solution was withdrawn at time intervals and tested for the presence of glucose. A control test was carried out without sample [15]. The glucose diffusion retardation index (GDRI) was calculated using the following formula.

$$\text{GDRI} = 100 - \left(\frac{\text{Glucose concentration in external solution in presence of extract}}{\text{Glucose concentration in external solution in absence of extract}}\right) \times 100$$

2.7 Statistical analysis

The results for all experiments were statistically analysed using a paired Student's t-test, with significance levels presented at $p< 0.05$.

3. Results

3.1 Physicochemical Analysis

The physicochemical test was conducted to assess whether high-amylose genotype like Kattuyanam rice, exhibit a hard or soft texture upon cooking. Kattuyanam rice was tested for its gel consistency and aroma, with the results summarized in Table 2. The gel consistency was determined to be 78.48, based on the length of the gel. For aroma detection, Kattuyanam rice produced a strong fragrance after being treated with potassium hydroxide for 15 minutes.

Table 2 Gel consistency

Genotype	Gel consistency value (mm)	Aroma
Kattuyanam	78.48±1.29	Strong

Values are mean ± standard deviation of three determinants.

3.2 Proximate analysis

The proximate composition analysis of Kattuyanam rice revealed a moisture content of 13.10 %, crude protein of approximately 9.64 g and crude lipid of 1.72 g. Additionally it contained 0.41 g of total ash, 1.14 g of crude fat, and 8.24 g of dietary fiber and Dietary fiber. These values (Table 3) highlighted the nutritional profile of Kattuyanam rice.

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Table 3 Proximate composition of the three rice genotypes

Genotype	Components (g/100g)					
	Moisture (%)	Crude Protein	Crude Lipid	Total ash content	Crude Fat	Dietary Fiber
Kattuyanam	13.10±0.24	9.64±0.10	1.72±0.02	0.41±0.04	1.14±0.06	8.24±0.03

Values are mean ± standard deviation of three determinations.

3.3 Total soluble sugars, starch, amylose and energy determination

The analysis of Kattuyanam rice showed total soluble sugars of 56 g/100 g, total starch content of 36 g/100 g, and an amylose content of 24.1 %. The energy value was calculated as 366.21 kcal per 100 g, reflecting its caloric contribution and carbohydrate profile (Table 4).

Table 4 Total soluble sugars, amylose content and energy values of the rice genotypes

Genotype	Total soluble sugars (g/100g)	Total starch (g/100g)	Amylose content (%)	Energy kcal $(100g)^{-1}$
Kattuyanam	56±0.13	36±0.12	24.1±0.04	366.21±2.2

Values are mean ± standard error of three determinations.

3.4 Quantitative analysis of Phytochemicals

The phytochemical analysis of Kattuyanam rice extracts revealed that the methanol extract contained higher concentrations of bioactive compounds compared to the ethanol extract (Table 5). Specifically, the anthocyanin content in the methanol extract was 213.21 mg/100g, while the ethanol extract had 181.14 mg/100g. Flavonoid content was also higher in the methanol extract, measured at 17.33 mg/mL, compared to 11.16 mg/ml in the ethanol extract. Additionally, the phenolic content was more prominent in the methanol extract, recorded at 161.4 µg GAE/g, in contrast to 124.5 µg GAE/gin the ethanol extract. These findings suggested that methanol is a more effective solvent for extracting these phytochemicals from Kattuyanam rice.

Table 5 Quantitative analysis of Phytochemicals

Extract	Anthocyanin (mg/100g)	Flavonoid (mg/mL)	Phenols (µg GAE/g)
Ethanol	181.14±0.05	11.16±0.09	124.5±1.7
Methanol	213.21±0.14	17.33 ± 0.11	161.4±3.8

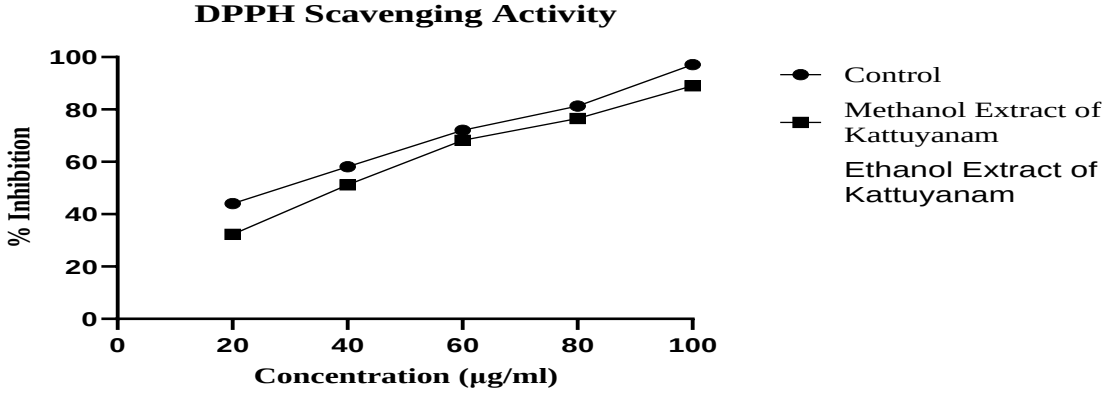
Values are mean ± standard error of three determinations.

3.5 Antioxidant analysis

3.5.1 DPPH radical scavenging activity

The DPPH radical scavenging activity analysis revealed the antioxidant potential of Kattuyanam rice extracts at various concentrations, highlighting the effectiveness of both ethanol and methanol extracts in inhibiting free radicals. The control exhibited a high inhibition of 97.13 % at 100 µg/mL, indicating the baseline antioxidant activity (Figure 1). In contrast, the methanol extract showed substantial activity, with an inhibition of 89.04 % at the same concentration, outperforming the ethanol extract, which reached only 53.45 % inhibition. Even at lower concentrations, the methanol extract consistently demonstrated superior scavenging abilities; for instance, at 80 µg/mL, it achieved 76.51 % inhibition, compared to just 41.06% for the ethanol extract. This suggested that the methanol extract of Kattuyanam rice possessed a stronger antioxidant potential than the ethanol extract, indicating its potential for health benefits related to oxidative stress.

Figure 1 DPPH Scavenging Activity of the Kattuyanam Rice Extract



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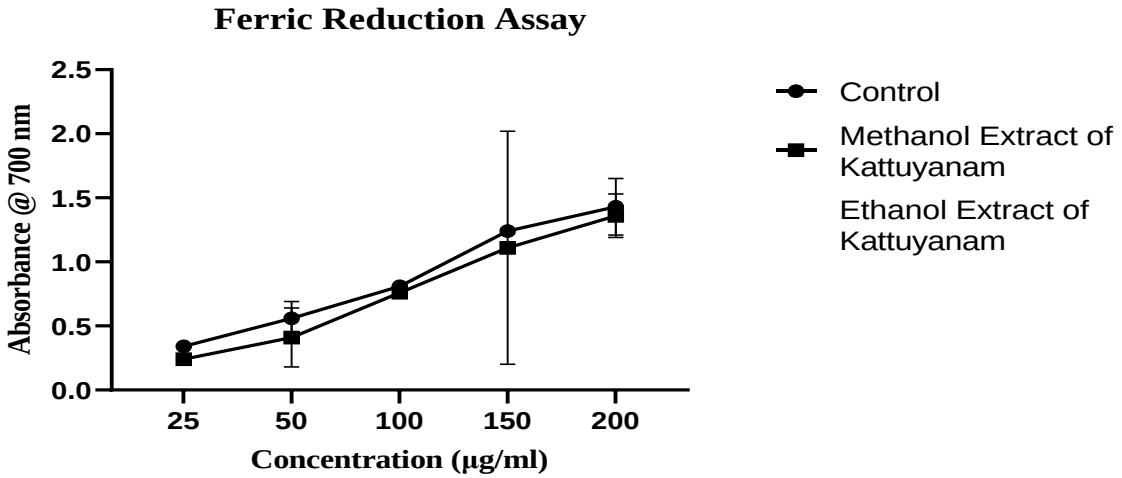
3.5.2 Ferric Reduction Assay

The ferric reduction assay was conducted to evaluate the reducing power of Kattuyanam rice extracts compared to the control at various concentrations. The control demonstrated increasing absorbance values with concentration, peaking at 1.43±0.22 at 200 µg/mL, indicating a strong reducing capability (Figure 2). Both the rice extracts showed lower absorbance compared to the control but managed to produce strong reduction capacity. At 200 µg/mL, the ethanol extract recorded an absorbance of 1.21±0.07, while the methanol extract reached 1.36±0.17, indicating their significant reducing activity as well. Notably the methanol extract exhibited a higher reducing power that the ethanol extract at concentrations of 100 and 150 µg/mL, with absorbance values of 0.76±0.02 and 1.11±0.14, respectively, while the ethanol extract only reached 0.52±0.03 and 0.91±0.01 at those concentrations. Statistical analysis revealed significant differences between the extracts and the control, particularly at higher concentrations ($p < 0.005$).

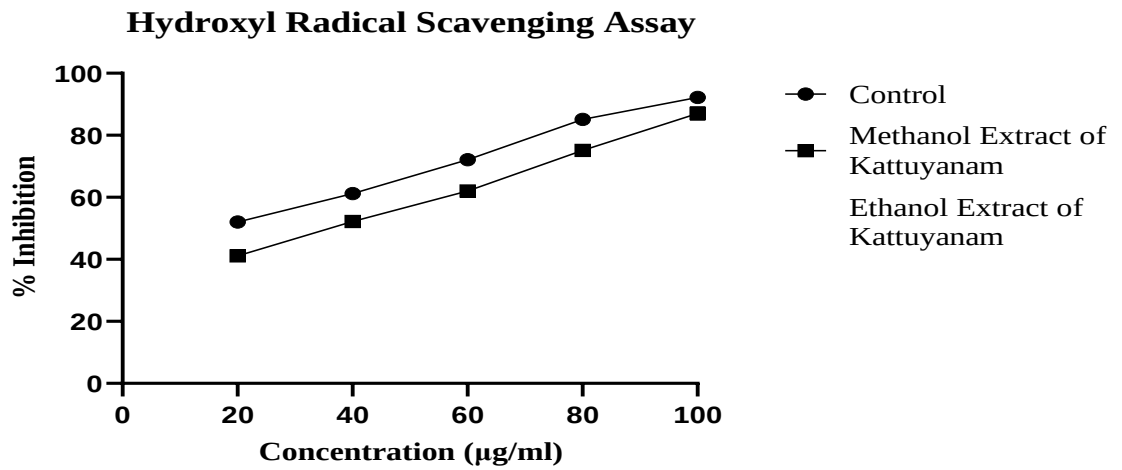
Figure 2 FRAP Assay of the Kattuyanam Rice Extracts



3.5.3 Hydroxyl Radical Scavenging Assay

The hydroxyl radical scavenging activity of Kattuyanam rice extracts was evaluated across various concentrations (20-100 µg/mL). The control group exhibited a % inhibition of 92.17±1.07 at the highest concentration of 100 µg/mL (Figure 3), indicating significant scavenging ability. The ethanol extract demonstrated a % inhibition of 72.11±0.72, while the methanol extract showed a higher inhibition of 87.05±2.13 at the same concentration, suggesting its superior efficacy. Both extracts exhibited a dose-dependent increase in scavenging activity, with the methanol extract consistently outperforming the ethanol extract at all tested concentrations, reflecting their potential antioxidant properties.

Figure 3 Hydroxyl Radical Scavenging Assay of the Rice Extracts



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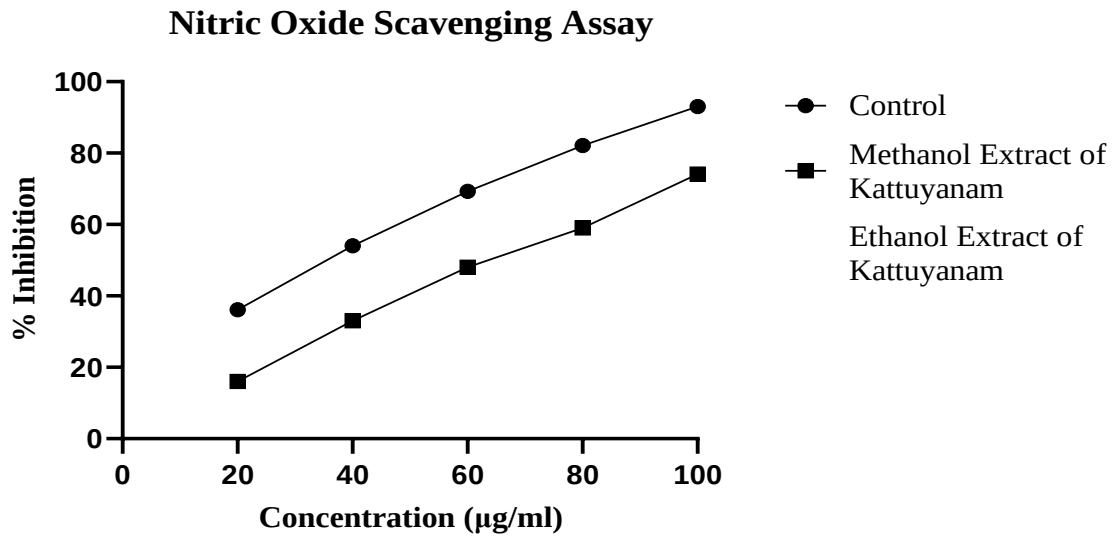
3.5.4 Nitric Oxide Scavenging Assay

The nitric oxide scavenging activity of Kattuyanam rice extracts was assessed across various concentrations (20 -100 µg/mL). The control group exhibited a significant inhibition of 93.03±1.01 % at 100 µg/mL (Figure 4). The ethanol extract demonstrated an inhibition of 62.08±0.04 % at the same concentration, indicating a notable but lower scavenging capability compared to the control. Conversely, the methanol extract showed a higher inhibition of 74.14±0.41 % at 100 µg/mL, suggesting it may be more effective scavenging nitric oxide than the ethanol extract. All extracts displayed a clear dose-dependent increase in inhibition, with higher concentrations yielding greater activity. No significant differences were observed among the treatments, emphasizing the antioxidant potential of both extracts.

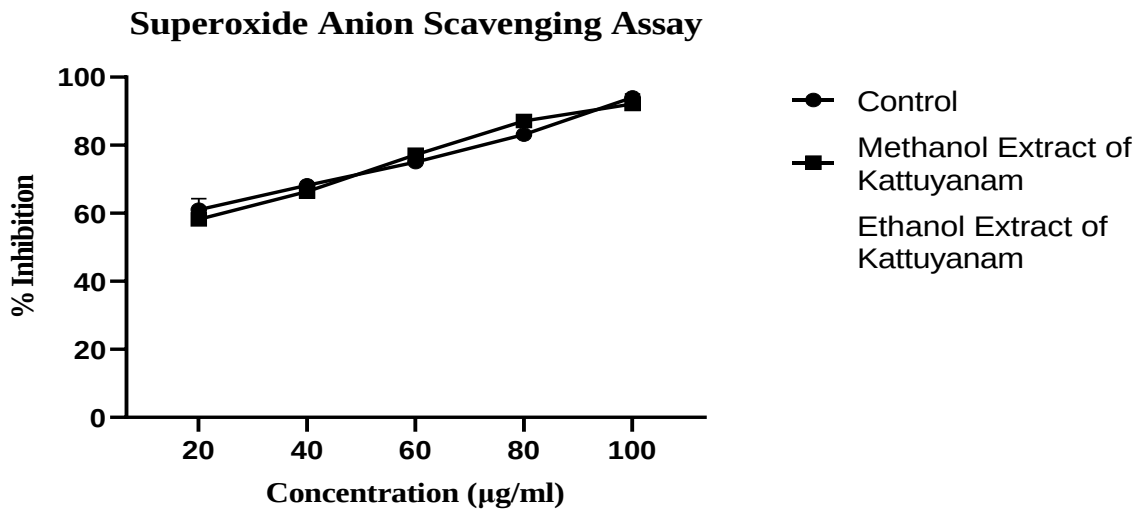
Figure 4 Nitric Oxide Scavenging Assay of the Rice Extracts



3.5.5 Superoxide Anion Scavenging Assay

The superoxide anion scavenging activity of Kattuyanam rice extracts was evaluated at various concentrations (20-100 µg/mL). The control group exhibited a high inhibition rate of 94.03±1.04 % at 100 µg/mL (Figure 5). Both the ethanol and methanol extracts showed notable scavenging effects, with the ethanol extract reaching an inhibition of 88.06±0.09 % and the methanol extract achieving a slightly higher inhibition of 92.11±0.78% at the same concentration. All extracts demonstrated a dose-dependent increase in inhibition, indicating their potential antioxidant properties. The differences among the treatment were statistically non-significant, suggesting similar scavenging capabilities of both extracts.

Figure 5 Superoxide Anion Scavenging Assay of the Rice Extracts



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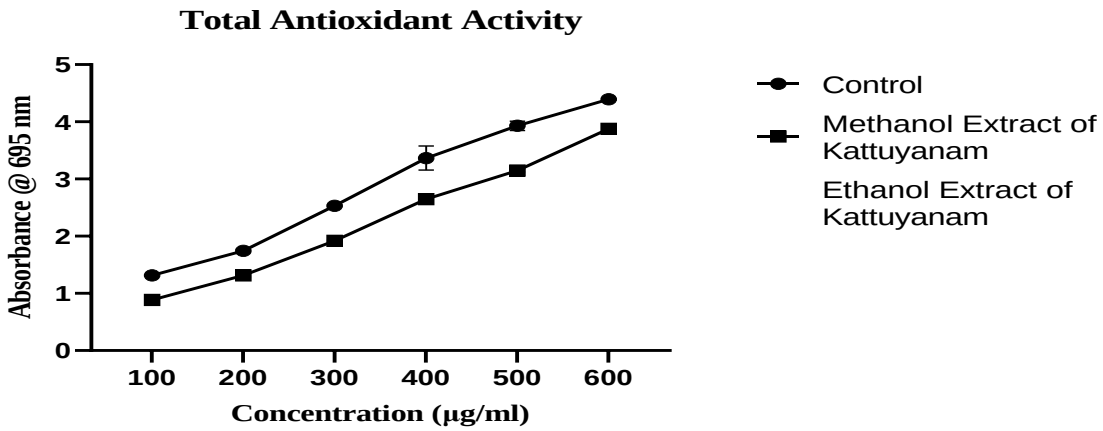
3.5.6 Total antioxidant activity

The total antioxidant activity of Kattuyanam rice extracts was assessed at varying concentrations (100-600 µg/mL) by measuring absorbance at 693 nm (Figure 6). The control group showed a maximum absorbance of 4.394±0.016 at 600 µg/mL. The ethanol extract exhibited lower absorbance values, reaching 2.821±0.017 at the same concentration, while the methanol extract demonstrated higher activity with an absorbance of 3.875±0.019. Significant differences were observed between the ethanol and methanol extracts, particularly at 400 µg/mL (1.982±0.019 for ethanol and 2.648±0.032 for methanol), indicating that the methanol extract may have superior antioxidant properties. Statistical significance was noted with $p<0.005$ for the two different rice extracts.

Figure 6 Total Antioxidant Activities of the Kattuyanam Rice Extracts

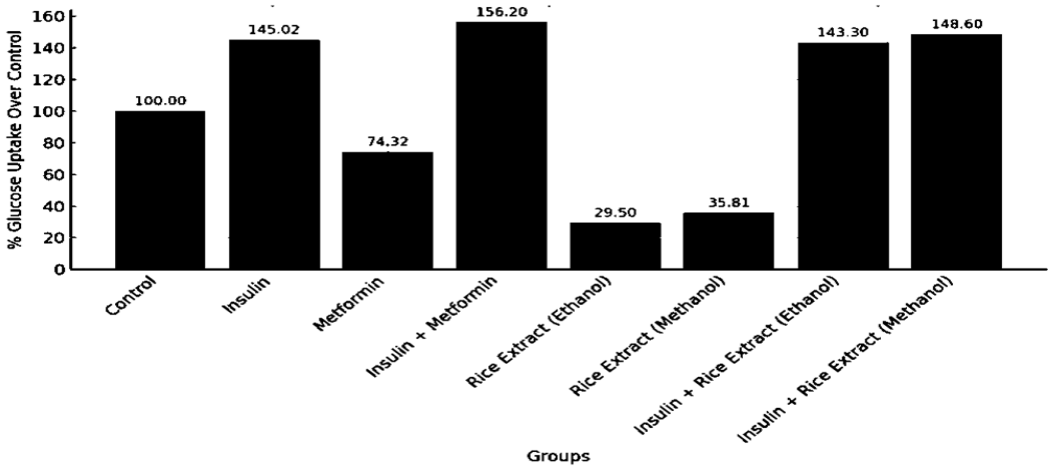


3.6 Invitro antidiabetic analysis

3.6.1 Glucose uptake Assay

The glucose uptake results from the various incubation mediums were depicted in Figure 7. The control group exhibited a baseline glucose uptake of 100.00%. The addition of insulin significantly increased glucose to 145.02%. In Contrast, metformin alone resulted in a lower uptake of 74.32%. When insulin and metformin were combined, glucose uptake further increased to 156.20%. Both ethanol and methanol exacts of rice showed comparatively low glucose uptake at 29.50% and 35.81% respectively. However, when combined with insulin, the rice extracts enhanced glucose uptake, with the ethanol extract resulting in 143.30% and the methanol extract reaching 148.60%. These findings indicate the potential of insulin to enhance glucose uptake significally, as well as a synergistic effect when combined with rice extracts.

Figure 7 Glucose Uptake Assay of the rice extracts



3.6.2 Glucose Adsorption Assay

The glucose adsorption capacity of Kattuyanam rice extracts was evaluated at various concentrations (5. 10, 15, 20 and 30 mM) as shown in Figure 8. The methanol extract consistently outperformed the ethanol extract in glucose adsorption across all tested concentrations. At 5 mM, the methanol extract demonstrated an adsorption of 4.82±0.202 mg/dL, while the ethanol extract was lower at 3.64±0.121 mg/dL. This trend continued at 10 mM, where the methanol extract showing

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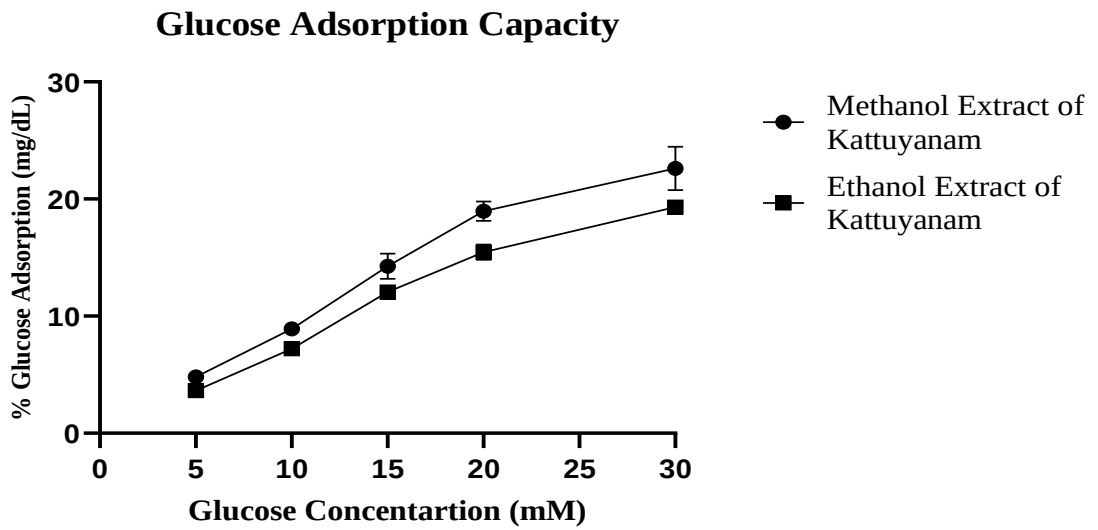
14.27±1.08 mg/dL versus 12.07±0.398 mg/dL for the ethanol extract. At 20 mM, the methanol extract reached 18.96±0.825 mg/dL, while the ethanol extract recorded 15.46±0.652 mg/dL. Finally, at 30 mM, the methanol extract exhibited an impressive capacity of 22.62±1.86 mg/dL compared to 19.32±0.415 mg/dL for the ethanol extract. The results suggested that Kattuyanam rice extracts, particularly methanol extract, have strong glucose adsorption potential, indicating their possible applications in glucose management.

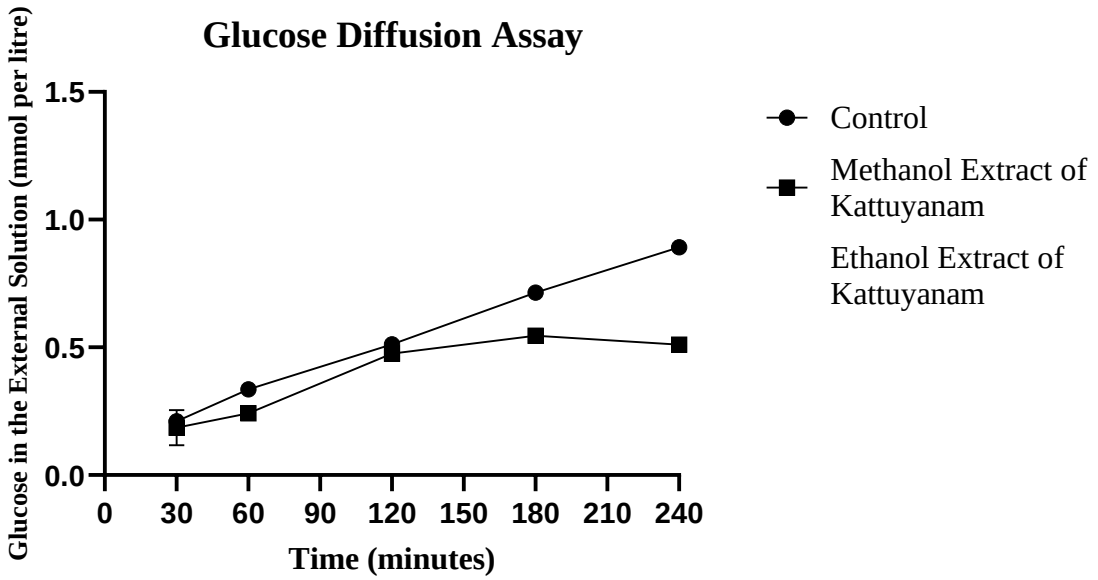
Figure 8 Glucose Adsorption capacity of the rice extracts



3.6.3 Glucose Diffusion Inhibition Assay

The glucose diffusion assay demonstrated varying degree of glucose concentration over time among the methanol extract, ethanol extract and control. At 60 minute, the methanol extract exhibited a lower glucose concentration of 0.242 mmol/L compared to 0.437 mmol/L for the control (Figure 8), indicating its initial effectiveness in reducing glucose diffusion. As time progressed to 180 minutes, the methanol extract still maintained a lower diffusion rate (0.546 mmol/L), while the ethanol extract and control reached 0.707 mmol/L and 0.714 mmol/L, respectively. By the end of the experiment (240 minutes), the methanol extract consistently showed the lowest glucose concentration (0.511 mmol/L), demonstrating its superior glucose-limiting ability when compared to the ethanol extract (0.965 mmol/L) and the control (0.892 mmol/L). This suggested that the methanol extract of Kattuyanam rice may have potential glucose-modulating properties over time.

Figure 8 Glucose Diffusion Assay of the rice extracts



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4. Discussion

Cereals are the major source of carbohydrates, proteins, minerals and vitamins to the world population. Gel consistency is an index that measures the tendency of the cooked rice to harden after cooling. Kattuyanam rice exhibited a higher gel consistency of 78.48 and hence it was considered as Soft gel consistency genotype. In general, gel lengths of more than 61 mm are considered as soft gel genotypes. In such context, Kattuyanam rice with a gel length higher than 61 mm could be considered as soft gel consistency genotypes. Rice with soft gel consistency is widely preferred by rice consumers. In the context of aroma, Kattuyanam rice produced a strong aroma after treatment with potassium hydroxide. Aroma is a key factor in determining the market value and is an important grain quality trait in rice. In the context of nutrition, Kattuyanam was found to be enriched with higher protein, dietary fibre content. Higher consumption of dietary fiber in particular cereal fiber reduces the risk or incidence of developing type 2 diabetes [16]. Hence Kattuyanam rice can be employed in the management of type 2 diabetes. From this proximate analysis, it could be made understood Kattuyanam rice to be highly nutritious apart from its therapeutic benefits.

Moreover, Kattuyanam rice was found to contain significantly lower levels of total soluble sugars and starch. Amylose content is an important characteristic that determines the quality of the rice. The total amylose content of Kattuyanam was found to be 24.2%. Popularly consumed rice varieties contains only 20% (approximately) of amylose content. Rice varieties with total amylose content greater than 20% are categorized as Group II [17]. Hence Kattuyanam rice could be classified under rice quality Group II. Anthocyanin, Flavanoid and Phenols present in Kattuyanam rice was quantified to evaluate its potential as nutritional function. In general, the pharmacological effect of a plant or cereal is mainly due to the presence of bioactive chemical present in it [18]. So it is very clear that it is the anthocyanin compound or phenolic compound or Flavanoid or any other plant compound that exerts the antioxidant and therapeutic values of the plant. With respect to anthocyanin, the methanol extract of Kattuyanam rice showed its presence in higher amount (213.21±0.14 mg/100g). The present research has shown that the methanol extract of Kattuyanam was found to harbour a higher content of flavonoids of17.33 ± 0.11mg/mL. The total phenolic content in methanol extract of Kattuyanam (161.4±3.8 µg GAE/g) was higher than ethanol extract. These evidences clearly depicts methanol extract of Kattuyanam rice to possess higher amounts of anthocyanins, phenols and flavonoids favouring its antioxidant and pharmacological potential.

The determination of the antioxidant activity is very crucial one since the antioxidants provides scavenging abilities against the free radicals generated due to auto-oxidation of glucose in diabetics [19]. The results from various antioxidant assays highlighted the significant antioxidant potential of Kattuyanam rice extracts, particularly when comparing ethanol and methanol extracts. In the DPPH radical scavenging activity assay, the methanol extract consistently demonstrated stronger free radical inhibition compared to the ethanol extract, achieving 89.04% inhibition at 100 µg/mL, while the ethanol extract reached only 53.45%. The ferric reduction assay showed that the methanol extract also outperformed the ethanol extract, particularly at higher concentrations, indicating superior reducing power. In the hydroxyl radical scavenging assay, the methanol extract again showed greater efficacy than the ethanol extract. Similarly, in the nitric oxide and superoxide anion scavenging assays, the methanol extract exhibited stronger inhibition of free radicals, consistently demonstrating dose-dependent activity. Finally in the total antioxidant activity assay, the methanol extract displayed higher absorbance at 600 µg/mL, indicating superior antioxidant capacity compared to the ethanol extract. Overall, the methanol extract of Kattuyanam rice consistently outperformed the ethanol extract across all assays, highlighting its potential as a more effective antioxidant source.

The *in vitro* antidiabetic analysis of Kattuyanam rice extracts demonstrated promising potential for glucose modulation, which may be beneficial in managing diabetes [20]. The glucose uptake assay revealed the insulin significantly increased glucose uptake. However, when combined with insulin, metformin further enhanced glucose uptake, illustrating the synergistic effects of these agents. Similarly the ethanol and methanol extracts of Kattuyanam rice showed low glucose uptake when administered individually but, in combination with insulin, both extracts significantly boosted glucose uptake. This synergy suggested that Kattuyanam rice extract may enhance the ability of the insulin to facilitate glucose absorption, with the methanol extract showing the most pronounced effect. In the glucose adsorption assay, Kattuyanam rice extracts, particularly the methanol extract, demonstrated strong glucose adsorption capacities across various concentrations. The methanol extract consistently outperformed the ethanol extract, suggesting a greater ability to limit glucose absorption in the bloodstream, which is crucial for controlling postprandial blood sugar spikes. This glucose adsorption property highlighted the potential of Kattuyanam rice, especially the methanol extract, as a dietary intervention to manage glucose levels in diabetic patients. The glucose diffusion inhibition assay further underscored the efficacy of the methanol extract, which showed the lowest glucose concentration over time compared to the ethanol extract and control. This suggest that the methanol extract may slow glucose diffusion, potentially delaying carbohydrate breakdown and glucose absorption in the gut. Such properties were essential for controlling glycemic load and improving glycemic control in individuals with diabetes. Overall, the findings indicated that Kattuyanam rice, particularly in its methanol extract form, exhibits significant antidiabetic properties through mechanisms such as enhancing glucose uptake, promoting glucose adsorption and inhibiting glucose diffusion.

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5. Conclusion

The present study on revealing the nutritional and antidiabetic potential of Kattuyanam rice revealed that it possessed significantly lower level of total soluble sugars, higher amount of dietary fibre and amylose. The nutritional profiling of this indigenous rice variety promotes its health benefits. The quantification of phytochemicals showed the presence of important bioactive compounds including phenols, flavonoids and anthocyanins in higher amount in the methanol extract of Kattuyanam rice. Further antidiabetic potential of the extracts including glucose diffusion inhibition and glucose adsorption and glucose uptake clearly reveals its therapeutic potential to be exploited in the management and treatment of Diabetes mellitus.

Declarations:

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