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Original Article

BIOACTIVE POTENTIAL OF NON-FERMENTED AND FERMENTED KULLAKAR RICE PORRIDGE: *IN VITRO* EVALUATION ON THE THERAPEUTIC PROPERTIES

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ABSTRACT

Kullakar rice, a traditional indigenous variety, is known for its rich nutritional and bio-functional properties. This study investigates the impact of fermentation on the phytochemical composition, antioxidant activity, and bio-functional potential of Kullakar rice flour porridge. Phytochemical, anti-oxidant and GC-MS was performed using constructive procedure. The anti-inflammatory potential was assessed using the protein denaturation inhibition assay, while the anti-diabetic potential was evaluated through alpha-amylase inhibition. The anti-cancer potential was determined using the MTT assay. Phytochemical for analysis was revealed that both fermented and non-fermented samples contained essential compounds, with fermentation significantly increasing total phenolic (12.14 ± 0.75 mg GAE/g) and total flavonoid (57.36 ± 0.34 mg QE/g) content while reducing tannins (185.75 ± 0.62 mg CE/g). Antioxidant activity was enhanced in the fermented porridge, as demonstrated by higher IC_{50} value of DPPH• radical scavenging activity (14.87 ± 0.76 µg/mL) and superoxide radical scavenging activity (18.87 ± 0.25 µg/mL) and improved phosphomolybdenum reduction activity (15.27 ± 0.15 µg/mL) and FRAP (40.62 ± 0.30 µg/mL) values. Bio-functional studies showed that fermentation strengthened IC_{50} value of anti-inflammatory (117.40 ± 1.05 µg/mL) and anti-diabetic (78.37 ± 0.50 µg/mL) properties. However, non-fermented porridge exhibited higher IC_{50} value of anti-cancer activity (5.27 ± 0.21 µg/mL), which was moderated upon fermentation. GC-MS analysis identified an increased presence of bioactive compounds in the fermented sample, further supporting its functional food potential. These findings suggest that fermentation enhances the nutritional and therapeutic benefits of porridge prepared using Kullakar rice flour, making it a promising candidate for health-focused dietary applications

KEYWORDS: Kullakar rice, Fermentation, Porridge, Anti-cancer, Anti-inflammatory

1. Introduction

Rice (*Oryza sativa* L.) is a staple grain cultivated across diverse climatic regions, from temperate to tropical zones, with Asia being a major producer. It serves as a primary food source for more than half of the global population [1]. Rice is available in various cultivars, distinguished by pericarp colour, including white, red, black, and purple [2]. Among the 40,000 rice varieties cultivated worldwide, pigmented rice stands out for its rich colours and nutritional value. Its hues range from deep purples and bright reds to striking blacks, blending tradition with modern culinary appeal [3]. Research on pigmented rice has primarily focused on the relationship between anthocyanins, antioxidants, and its nutritional properties. Red rice, in particular, contains higher levels of proanthocyanidins and other phenolic compounds [4, 5]. It demonstrates greater antioxidant activity compared to non-pigmented varieties, with the intensity of pigmentation correlating to higher flavonoid content and stronger antioxidant properties [6,7,8]. The health benefits of anthocyanins, including antioxidant, anti-inflammatory, and anti-carcinogenic properties, coloured rice is recognized as a functional food and ingredient in many Asian countries. The health benefits of anthocyanins present in red and black rice varieties have been well-documented, highlighting their nutritional significance [9]. An addition to the anthocyanins

pigmented rice is gaining popularity for its rich bioactive compounds, including phenols, flavonoids, essential minerals, vitamins, and plant sterols, which contribute to its nutritional benefits [10,11,12]. Red rice varieties are rich in phenolc compounds, including ferulic acid, p-coumaric acid, and vanillic acid [6], as and research indicates that p-coumaric acid and vanillic acid play a significant role in the antioxidant properties of red rice [13].

Kullakar is a traditional rice variety from Tamil Nadu, India, with an unknown pedigree. It has a growth duration of 120–125 days and reaches an average height of 110 cm. The variety produces 100–110 grains per ear head and yields approximately 1400 kg per acre. The 1000-grain weight is 25 grams, and the pericarp is red in colour [14]. Research shows that Kullakar rice helps reduce body mass index and functions as a cardiotonic [15]. Among many preparations rice porridge is a traditional dish in many Asian countries, including Thailand, China, Japan, Singapore, and the Philippines [16]. It is commonly served to individuals with digestive issues, patients with reduced appetite, and the elderly with swallowing difficulties [17]. Fermented food is produced through microbial fermentation, which significantly alters its flavour and quality. Grains, a staple food source, can undergo fermentation to create various products such as bread, alcoholic beverages, condiments, and fermented porridge. Fermented porridge has a long history and exists in diverse forms across different cultures [18]. Traditional fermented foods are the ideal source of novel probiotic isolates which are known to have significant therapeutic benefits and play a vital role as bioprotective agents [19]. This study aims to evaluate the bioactive properties of fermented and non-fermented Kullakar rice flour porridge. The research focuses on phytochemical analysis, estimation of phenols and flavonoids, assessment of antioxidant potential through various assays, and the evaluation of in-vitro anti-inflammatory, anti-diabetic, and anti-cancer activities. Additionally, Gas Chromatography-Mass Spectrometry (GC-MS) analysis will be performed to identify key phytoconstituents present in the samples. The findings will provide insights into the health benefits of fermented and non-fermented Kullakar rice flour porridge and its significance in promoting well-being.

2. Materials and Methods

The study protocol was reviewed and approved by the Institutional Ethics Committee (No. WCC/IEC/2024:135).

2.1. Chemicals and Reagents

All chemicals used were of analytical grade. Dragendorff's reagent, ammonium hydroxide, methanol, and acetic anhydride were obtained from SRL (Sisco Research Laboratories). Ferric chloride, aluminium chloride, and sodium carbonate were procured from Thermo Fisher Scientific India Pvt. Ltd., while chloroform, sodium nitrate, and gallic acid were purchased from LOBA Chemie Pvt. Ltd. Concentrated sulfuric acid, glacial acetic acid, and DPPH were sourced from Nice Chemicals. Riboflavin and other antioxidant reagents like EDTA and ascorbic acid were obtained from Merck Ltd. and Sisco Research. MTT for cytotoxicity assay was obtained from Invitrogen (USA), and other anti-cancer reagents including RPMI, FBS, and antibiotics were procured from Sigma-Aldrich, Qualigens Fine Chemicals, SRL, and Nice Chemicals.

2.2. Procurement of sample

The raw Kullakar rice sample was collected from organic vendor namely Iyarkai Virumbi Angadi located in Vadaloor village, Tamil Nadu, India.

2.3. Preparation of non-fermented Kullakar rice flour porridge extract

The rice sample was washed thrice, soaked in water for 3 hours, and sun-dried for 2 days. The dried Kullakar rice was milled into fine powder and sifted to remove impurities. To prepare porridge, 10 grams of rice powder was mixed with 15 ml of water, boiled for 8–10 minutes and cooled. To prepare the extract, 5 ml of the non-fermented porridge was soaked in 50 ml of ethanol for 3 days, and the extract was analyzed

2.4 Preparation of fermented Kullakar rice flour porridge extraction

The rice sample was washed thrice, soaked for 3 hours and sun-dried for 3 days. The dried rice was milled into fine flour and sifted. Ten grams of Kullakar rice flour was mixed with 15 ml of water, added to boiling water, and cooked for 10 minutes. After overnight fermentation, to prepare extract 5 ml of porridge was soaked in 50 ml of ethanol for 3 days, and the extract was analyzed.

2.5 Phytochemical analysis of fermented and non-fermented Kullakar rice flour porridge

The following test were performed to explore the phytochemical characteristic in fermented and non-fermented Kullakar rice flour porridge. And to detect the various phytoconstituents present in it. The procedure for the phytochemical screening is as follows:

2.5.1 Detection of Alkaloids

Approximately 0.5 ml of the sample extract was taken and combined with a few drops of concentrated hydrochloric acid, ensuring thorough mixing. Subsequently, a few drops of Dragendorff's reagent were added. The appearance of an orange or brown color indicated the presence of alkaloids [20].

2.5.2 Detection of Tannins

For the detection of tannins, 0.5 ml sample of porridge was diluted in 20 ml of distilled water in a test tube and then filtered. To the filtrate, 0.1% $FeCl_3$ was added, and the resulting colour change was observed. The appearance of a brownish-green or blue-black coloration indicated the presence of tannins [20].

2.5.3 Detection of Saponins

For the detection of saponins, 2 ml of porridge extract was first diluted in 20 ml of distilled water and filtered. From the filtrate, 1 ml was combined with 5 ml of distilled water in a separate test tube and shaken vigorously to produce a stable froth. Three drops of olive oil were then added, and the formation of an emulsion confirmed the presence of saponins [20].

2.5.4 Detection of Flavonoids

A few drops of 1% NH_3 solution were added to the test tube containing the sample. The appearance of a yellow coloration indicated the presence of flavonoid compounds [20].

2.5.5 Detection of Terpenoids

In separate test tubes, 1 ml of each porridge sample was mixed with 400 µL of $CHCl_3$. To this mixture, 600 µL of concentrated H_2SO_4 was carefully added to form a distinct layer. The formation of a reddish-brown interface indicated the presence of terpenoid constituents [20].

2.5.6 Detection of Glycosides

For the detection of glycosides, 500 µL of concentrated H_2SO_4 was prepared in a test tube. Then, 2 ml of each porridge sample was mixed with 1 ml of glacial CH_3CO_2H containing one drop of $FeCl_3$. This mixture was carefully layered over the concentrated H_2SO_4, ensuring the H_2SO_4 remained beneath the mixture. The appearance of a brown ring at the interface indicated the presence of glycoside constituents [20].

2.5.7 Detection of Phenolic compounds

The sample of 0.5 ml was dissolved in 5 ml of distilled water and few drops of neutral 5% ferric chloride solution were added. A dark green/violet colour indicated the presence of phenolic compounds [20].

2.5.8 Detection of steroids

A few drops of concentrated sulfuric acid were added to 3 ml of porridge extract, followed by acetic anhydride. The development of a brown to red colour indicated a positive result for steroids, as reported by Jeba et al. [21].

2.6. Estimation of total phenols and total flavonoids of fermented and non-fermented Kullakar rice flour porridge

The extract of fermented Kullakar rice flour porridge and non-fermented Kullakar rice flour porridge were examined to find the total content of phenols, flavonoids and tannin

2.6.1 Determination of total phenolic content (TPC)

Total phenolic content in porridge extracts was determined using the Folin-Ciocalteu reagent, with gallic acid as the standard. A 0.5 ml sample was mixed with 1 ml of 10-fold diluted reagent and 1 ml of 7.5% sodium carbonate. After 30 minutes at room temperature, absorbance was measured at 760 nm using a UV-Spectrophotometer [22]

2.6.2 Determination of total flavonoid content (TFC)

The total flavonoid content (TFC) of the porridge extract was determined using the aluminum chloride colorimetric method, with quercetin as the standard. For the assay, 1 ml of 5% sodium nitrate was added, followed by 1 ml of 10% aluminum chloride. After 5 minutes of incubation, 1 ml of 1 M NaOH was added, and the solution was diluted with 2.4 ml of distilled water to a final volume of 10 ml. The mixture was homogenized, and absorbance was measured at 533 nm using a UV-Spectrophotometer. TFC was calculated using a quercetin standard curve and expressed as quercetin equivalents (mg/g) [23].

2.6.3 Determination of total tannin content

Tannins were quantified using the Folin-Ciocalteu method. A volume of 0.1 ml of the sample extract was mixed with 7.5 ml of distilled water, 1 ml of 10% Folin-Ciocalteu reagent, and 1 ml of 35% sodium carbonate solution. The mixture was thoroughly shaken, left to stand at room temperature for 30 minutes, and its absorbance was measured at 725 nm. A blank was prepared by replacing the sample with water. Standard solutions of gallic acid were similarly prepared and analyzed, with their absorbance measured against the blank. The tannin content is reported as milligrams of gallic acid equivalent per gram of extract (mg GAE/g) [24].

2.7 Determination of anti-oxidant activity in fermented and non-fermented Kullakar rice flour porridge

The extracts of fermented and non-fermented Kullakar rice flour porridge were used to assess the antioxidant potential. The methods as follows.

2.7.1 DPPH• radical scavenging activity

The DPPH• (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity assay was conducted following the method described by Noipa et al., with slight modifications for enhanced accuracy. Stock solutions of the porridge extract were prepared at a concentration of 1 mg/mL and subsequently diluted with ethanol to obtain final concentrations of 20, 40, 60, 80, 100, and 120 µg/mL [25]. To each sample solution, 1 mL of a 0.3 mM DPPH• solution in ethanol was added. The DPPH• solution, containing a stable free radical, interacts with antioxidant compounds in the sample, leading to a reduction in the DPPH• radical. The reaction mixtures were incubated in the dark at room temperature for 30 minutes to prevent light-induced degradation of DPPH•. Following incubation, the absorbance of each solution was measured at 520 nm using a UV spectrophotometer. The antioxidant activity (AA) was expressed as a percentage reduction, calculated using the appropriate formula.

$$\text{Percentage of antioxidant activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{X100}$$

2.7.2 Superoxide radical scavenging activity

Superoxide radical scavenging activity was evaluated using the riboflavin-EDTA-NBT method, as described by Zhu et al. [26]. The reaction mixture included varying concentrations (20, 40, 60, 80, 100, and 120 µg/mL) of fermented and non-fermented Kullakar rice flour porridge extracts, each prepared in separate test tubes along with a control. To each test tube, 1 mL of methanol was added, followed by the sequential addition of 200 µL of 1.5 mM riboflavin solution, 100 µL of 12 mM EDTA solution, and 50 µL of 50 mM Nitroblue Tetrazolium (NBT) solution. The reaction was initiated by exposing the mixture to a light source for 90 seconds, inducing riboflavin photoreduction and generating superoxide radicals. Immediately after light exposure, absorbance was measured at 590 nm using a UV spectrophotometer. A decrease in absorbance indicated higher scavenging activity of the sample. Ascorbic acid was used as a positive control, and the results were analyzed to determine the superoxide radical scavenging

potential of both fermented and non-fermented Kullakar rice flour porridge extracts.

$$\text{Percentage of superoxide radical inhibition}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{X100}$$

2.7.3 Phosphomolybdenum reduction assay

The antioxidant capacity of the porridge extract was assessed using the Molybdenum (VI) reduction method, as described by Jeba et al. [21]. Different concentrations of the extract (20, 40, 60, 80, 100, and 120 µg/mL) were prepared by dissolving them in 1 mL of methanol in separate test tubes. To each tube, 1 mL of Phosphomolybdenum reagent was added. A control sample, prepared without the extract, was included to serve as a baseline for comparison. The reaction mixtures were incubated in a water bath at 95°C for 30 minutes, allowing the formation of a colour complex indicative of antioxidant activity. Following incubation, the absorbance of the resulting complex was measured at 695 nm using a UV spectrophotometer. Ascorbic acid, known for its strong antioxidant properties, was used as the standard reference. The antioxidant capacity of the porridge extract was expressed as a percentage reduction in absorbance, calculated using the appropriate formula.

$$\text{Percentage of phosphomolybdenum reduction}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{X100}$$

2.7.4 Ferric (Fe^{3+}) reducing power assay

The antioxidant capacity of the extract was assessed using the potassium ferricyanide reducing power assay, as described by Jeba et al. [21]. For the assay, 1 mL of 1% potassium ferricyanide solution and 1 mL of phosphate buffer (pH 6.6) prepared in methanol were combined with 1 mL of the extract at varying concentrations (20, 40, 60, 80, 100, and 120 µg/mL). The reaction mixtures were incubated in a water bath at 50°C for 30 minutes to facilitate the reduction process. Following incubation, 500 µL of 10% trichloroacetic acid (TCA) was added to terminate the reaction, and 100 µL of freshly prepared ferric chloride ($FeCl_3$) solution was introduced to develop the color. The mixtures were thoroughly homogenized before measuring absorbance at 700 nm using a UV spectrophotometer. A higher absorbance indicated greater antioxidant activity. Data analysis was performed using GraphPad PRISM 10 software. Ascorbic acid was used as the standard reference, and the antioxidant activity of the samples was expressed as a percentage of reduction, calculated using the appropriate formula.

$$\text{Percentage of ferric (Fe3 +) reducing}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{X100}$$

2.8 Determination of *in-vitro* Anti-inflammatory activity of fermented and non-fermented Kullakar rice flour porridge

The extract of fermented Kullakar rice flour porridge and non-fermented Kullakar rice flour porridge was examined to find the anti-inflammatory activity using Bovine serum albumin denaturation method.

Bovine serum albumin denaturation method

The anti-inflammatory activity of porridge extracts was evaluated using a modified bovine serum albumin (BSA) assay, as described by Bailey-Shaw et al. [27]. Each 3 mL reaction mixture contained 50 µL of the test extract at different concentrations (100, 200, and 400 µg/mL) and 450 µL of a 5% w/v BSA solution prepared in Tris-buffered saline. To ensure reliability, distilled water served as the negative control, while diclofenac sodium (prepared in methanol) was used as the positive control, both subjected to the same experimental conditions as the test samples. The reaction mixtures were first incubated at 37°C for 20 minutes, followed by heating at 57°C for 3 minutes to induce protein denaturation. After cooling to room temperature, 2.5 mL of phosphate-buffered saline (pH 6.3) was added to stabilize the reaction. The turbidity, indicating the extent of protein precipitation and thereby anti-inflammatory activity, was measured using a UV spectrophotometer at 660 nm. Enzyme inhibition was then calculated accordingly.

$$\text{Percentage of protein denaturation}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{X100}$$

2.9 Determination of *in-vitro* Anti-diabetic activity of fermented and non-fermented Kullakar rice flour porridge

The extracts of fermented Kullakar rice flour porridge and non-fermented Kullakar rice flour porridge was examined to assess the anti-diabetic activity using alpha-amylase inhibition activity

Alpha-Amylase inhibition activity

The α-amylase inhibition activity of the selected extracts was assessed using the method of Ferosekhan et al. with slight modifications. Extracts at concentrations of 20, 40, 60, 80, 100, and 120 µg were prepared, and methanol was added to each tube to adjust the volume to 1 ml [28]. Then, 20 µL of 1% α-amylase solution and 1 ml of phosphate buffer were added, followed by incubation at 37°C for 5 minutes. After incubation, 200 µL of 1% starch solution was added, and the samples were further incubated at room temperature for 60 minutes. To stop the enzymatic reaction, 100 µL of 1 M HCl and 200 µL of iodine reagent were added. The mixtures were thoroughly shaken, and absorbance was measured at 595 nm using a UV spectrophotometer exactly 1 minute after adding the iodine reagent. The percentage of enzyme inhibition was calculated using the following formula.

$$\text{Percentage of inhibition}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{X100}$$

2.10 Determination of *in-vitro* Anti-Cancer activity of fermented and non-fermented Kullakar rice flour porridge

The extracts of fermented Kullakar rice flour porridge and non-fermented Kullakar rice flour porridge was examined to assess the anti-cancer activity using MTT assay.

Cytotoxicity and anticancer activity

The selected extracts were evaluated for anti-cancer activity using a modified version of the method described by Mosmann [29].

Cell Culture: HT29 cells, obtained from the National Centre for Cell Science (NCCS), Pune, were cultured in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% fetal bovine serum (FBS), along with penicillin/streptomycin (250 U/mL), gentamycin (100 µg/mL), and amphotericin B (1 mg/mL) from Sigma Chemicals, MO, USA. The cells were maintained at 37°C

in a humidified atmosphere with 5% CO_2 and allowed to reach confluence over 24 hours before experimentation

MTT Assay for Cell Growth Inhibition: Cell viability was assessed using a modified MTT reduction assay. The MTT assay is commonly performed as a preliminary experiment using a broad range of concentrations, often through serial dilution, to establish a suitable range where the assay is sensitive to changes in cell viability. This approach helps determine the dose-response relationship of a substance, indicating its cytotoxicity, and to identify the concentration that inhibits 50% of cell growth (IC_{50}). Negative control had cell viability 100%, which means without test sample, while the standard (Doxorubicin) was the positive control.

HT29 cells were seeded at a density of 5×10^3 cells per well in 96-well plates and cultured in 200 µL of RPMI medium supplemented with 10% FBS for 24 hours. After incubation, the culture medium was removed and replaced with RPMI medium containing varying concentrations (0.195–100 µg/mL) of the test extract. The cells were then incubated for an additional 48 hours.

Following treatment, 10 µL of MTT solution (5 mg/mL) was added to each well, and the plate was incubated at 37°C for 4 hours. The medium was then removed, and DMSO was added to dissolve the formazan crystals, followed by a 1-hour incubation at room temperature. Absorbance was measured at 595 nm using a multi-well spectrophotometer. The percentage of cell viability was calculated using the following formula.

$$\text{Percentage of cell viability}(\%) = \frac{\text{Mean OD}}{\text{Control OD}} \text{X}100$$

2.11 Statistical Analysis

Data analysis was performed using GraphPad PRISM 10 software. Results are presented as the mean and standard deviation of three independent experiments. One way ANOVA was used to study the difference in the in vitro activities of the standard, fermented Kullakar rice flour porridge and non-fermented Kullakar rice flour porridge. A p value of <0.05 was considered to be statistically significant.

2.12 Determination of Phytoconstituents using Gas Chromatography-Mass Spectrometry Analysis (GC-MS)

The bioactive compounds in porridge were analyzed using gas chromatography-mass spectrometry (GC-MS). Separation was performed on a 30 m × 0.25 mm i.d. Elite-5MS capillary column with a 0.25 µm film thickness. The sample, contained in a 22 ml headspace vial, was heated to 90 °C for 10 minutes before the gas phase was injected into the GC-MS. The injection time was 0.10 min in constant mode. GC conditions were as follows: the injector was set to 280 °C in split mode (10:1), and the column oven temperature started at 60 °C for 1 min, then increased by 4 °C/min to 280 °C, where it was held for 5 min. Helium served as the carrier gas at a constant flow rate of 1 ml/min. MS detection was conducted at 200 °C in electron impact (EI) mode, with full scan analysis from m/z 30 to 600 at a low scanning speed. Volatile compounds were identified by comparing their GC retention time and mass spectra with reference spectra from the US National Institute of Standards and Technology (NIST 2017) database, ensuring a similarity of over 75% [30].

3. Results

3.1 Qualitative Phytochemical Profile

Qualitative phytochemical profile of fermented and non-fermented Kullakar rice flour porridge is presented in table 1. Various phytochemicals, namely tannins, saponins, flavonoids, terpenoid glycosides, phenolic compounds, and steroids were present in both fermented and non-fermented Kullakar rice flour porridge samples. Notably, alkaloids were detected only in the non-fermented sample, while they were absent in the fermented porridge.

Table 1. Presence of phytochemical in fermented and non-fermented Kullakar rice flour porridge

Phytochemical	Presence/Absence	
	Fermented	Non-fermented
Alkaloid	-	+
Tannins	+	+
Saponins	+	+
Flavonoids	+	+
Terpenoid	+	+
Glycosides	+	+
Phenolic compounds	+	+
Steroids	+	+

+ Presence; - Absence

3.2 Quantitative Phytochemical Profile

The phytochemical composition of phenols, flavonoids, and tannins in non-fermented and fermented Kullakar rice flour porridge is presented in fig 1. Fermentation leads to a notable increase in total phenolic content, rising from 6.64 ± 0.34 µg/mg GAE in non-fermented porridge to 12.14 ± 0.75 µg/mg GAE. Similarly, total flavonoid content shows a slight increase from 54.59 ± 0.58 µg/mg QE in non-fermented porridge to 57.36 ± 0.34 µg/mg QE in fermented porridge. In contrast, total tannin content decreases from 244.76 ± 0.39 µg/mg TAE in non-fermented porridge to 185.75 ± 0.62 µg/mg TAE after fermentation.

3.3 Antioxidant activity

Antioxidant activity of fermented Kullakar rice flour and Non-fermented Kullakar rice flour porridge.

Table 2. IC_{50} value of Anti-oxidant of standard, fermented Kullakar rice flour porridge and Non-fermented Kullakar rice flour porridge

IC_{50} value	DPPH•	SRSA	PRA	FRPA
Standard (µg/mL)	6.07± 0.15	6.73± 0.15	4.50± 0.10	3.47± 0.15
FKRFP (µg/mL)	14.87± 0.76	18.87± 0.25	15.27± 0.15	40.50± 0.30
NFKRFP (µg/mL)	120.27± 1.27	68.23± 1.80	76.97± 0.74	113.97± 0.67
p-value	p<0.000	p<0.000	p<0.000	p<0.000

FKRFP - Fermented Kullakar rice flour porridge; NFKRFP - Non-fermented Kullakar rice flour porridge; DPPH- DPPH• radical scavenging activity; SRSA - Superoxide radical scavenging activity; PRA- Phosphomolybdenum reduction activity; FRPA - ferric (Fe^{3+}) reducing power activity

3.3.1 DPPH• radical scavenging activity

The percentage grows progressively with increasing concentration for DPPH• radical scavenging activity of fermented and non-fermented Kullakar rice flour porridge, the percentage shows a steady increase with rising concentration is presented in fig 2. The IC_{50} value of standard, fermented Kullakar rice flour porridge and non-fermented Kullakar rice flour porridge is presented in table 2 DPPH• radical scavenging activity. The IC_{50} of the fermented Kullakar rice flour porridge (14.87± 0.76 µg/mL) is higher than the standard ascorbic acid (6.07± 0.15 µg/mL) and possess radical-scavenging activity, suggesting that it is a potent natural antioxidant. Conversely, the non-fermented porridge shows a much greater IC_{50} value (120.27± 1.27 µg/mL), compared to the standard which reflects much lower DPPH• radical-scavenging activity. The fermented porridge is considerably superior to the non-fermented porridge, with a much lower IC_{50} value, reflecting the beneficial effect of fermentation in improving antioxidant activity ($p<0.000$).

3.3.2 Superoxide radical scavenging activity (SRSA)

Increased concentration of sample leads to a measurable percentage gain for superoxide radical scavenging activity of fermented and non-fermented Kullakar rice flour porridge (fig 3). The IC_{50} value of standard, fermented Kullakar rice flour porridge and non-fermented Kullakar rice flour porridge is presented in table 2 for superoxide radical scavenging activity. The IC_{50} of fermented Kullakar rice flour porridge (18.87± 0.25 µg/mL) is higher than the standard ascorbic acid (6.73± 0.15 µg/mL). The IC_{50} of non-fermented Kullakar rice flour porridge (68.23± 1.80 µg/mL) is also much higher than the standard. On comparison, the fermented porridge has better antioxidant activity than the non-fermented porridge ($p<0.000$).

3.3.3 Phosphomolybdenum reduction activity (PRA)

An increase in concentration results in a corresponding percentage rise for phosphomolybdenum reduction activity of fermented and non-fermented Kullakar rice flour porridge is presented in the fig 4. The IC_{50} value of standard, fermented Kullakar rice flour porridge and non-fermented Kullakar rice flour porridge is presented in table 2 for phosphomolybdenum reduction activity. Fermented Kullakar rice flour porridge has an IC_{50} value of 15.27± 0.15 µg/mL, non-fermented Kullakar rice flour porridge has an IC_{50} value of 76.97± 0.74 µg/mL. Both fermented and non-fermented are greater than the standard ascorbic acid (4.50± 0.10 µg/mL). However, fermented Kullakar rice flour porridge show effect than non-fermented, but both are less effect. This indicates that both fermented and non-fermented Kullakar rice flour porridge have reduction scavenging activity, but compared to both, fermented Kullakar rice flour porridge had higher reduction scavenging activity. The fermentation process significantly increases the reduction activity of Kullakar rice flour porridge, thus a more effective natural antioxidant compared to non-fermented rice flour porridge ($p<0.000$).

3.3.4 Ferric (Fe^{3+}) reducing power activity (FRPA)

The percentage shows a steady increase with rising concentration for ferric (Fe^{3+}) reducing power activity of fermented and non-fermented Kullakar rice flour porridge is presented in fig 5. The IC_{50} value of standard, fermented Kullakar rice flour porridge and non-fermented Kullakar rice flour porridge is presented in table 2 for ferric (Fe^{3+}) reducing power activity. The IC_{50} value of fermented Kullakar rice flour porridge (40.50± 0.30 µg/mL) is greater than standard ascorbic acid (3.47± 0.15 µg/mL). This indicates that the fermented porridge possesses lesser reducing power but exhibits good antioxidant activity and the non-fermented porridge also exhibits much greater IC_{50} value (113.97± 0.67 µg/mL) than standard, which clearly shows that both Kullakar rice flour porridge has weaker reducing power. Interestingly, when compare to both the IC_{50} value of fermented porridge is significantly lower compared to the non-fermented one, implying that fermentation maximally enhances antioxidant capacity of porridge ($p<0.000$).

3.4 In vitro studies of anti-inflammatory, anti-diabetic and anti-cancer

In Vitro studies like anti-inflammatory, antidiabetic and anti-cancer of fermented Kullakar rice flour porridge and Non-fermented Kullakar rice flour porridge.

3.4.1 Anti-inflammatory activity

The anti-inflammatory potential of fermented and non-fermented Kullakar rice flour porridge, is measured through protein denaturation inhibition. A higher concentration leads to a proportional rise in percentage for protein denaturation inhibition of fermented and non-fermented Kullakar rice flour porridge is presented in the fig 6. The IC_{50} value of standard, fermented Kullakar rice flour porridge and non-fermented Kullakar rice flour porridge is presented in table 3. The fermented Kullakar rice flour porridge has a lower IC_{50} value of 117.40± 1.05 µg/mL than the standard Diclofenac Sodium, which is 128.50± 0.66 µg/mL. This range suggests that fermented Kullakar rice flour porridge may be utilized as a natural food grade material with lesser chances of causing protein denaturation. In contrast, the non-fermented Kullakar rice porridge has an IC_{50} value of 366.33± 1.58 µg/mL, indicating that the inhibition has a lower potency ($p<0.000$).

Table 3. IC_{50} value of anti-inflammatory, anti-diabetic and anti-cancer of standard, fermented Kullakar rice flour porridge and Non-fermented Kullakar rice flour porridge

IC_{50} value	Anti-inflammatory	Anti-Diabetic	Anti-Cancer
Standard	128.50± 0.66	54.70± 1.23	11.63± 0.87
FKRFP	117.40± 1.05	78.37± 0.50	21.27± 1.15
NFKRFP	366.33± 1.58	119.79± 0.60	5.27± 0.21
p-value	p<0.000	p<0.000	p<0.000

FKRFP - Fermented Kullakar rice flour porridge; NFKRFP - Non-fermented Kullakar rice flour porridge

3.4.2 Anti- Diabetic activity

The anti-diabetic potential of fermented and non-fermented Kullakar rice flour porridge, measured through alpha-amylase inhibition. The percentage elevates as the concentration becomes higher for anti-

diabetic activity of fermented and non-fermented Kullakar rice flour porridge is presented in fig 7. The IC_{50} value of standard, fermented Kullakar rice flour porridge and non-fermented Kullakar rice flour porridge is presented in table 3. reveals a significant improvement after fermentation. The IC_{50} value of fermented porridge (78.37 ± 0.50 µg/mL) is lower than that of the non-fermented porridge (119.79 ± 0.60 µg/mL), indicating enhanced enzyme inhibition. Although the standard Acarbose (54.70 ± 1.23 µg/mL) exhibits greater potency, the fermented porridge still demonstrates notable inhibitory activity, suggesting its potential as a natural dietary option for managing postprandial blood sugar levels ($p<0.000$).

3.4.3 Anti-Cancer activity

The anti-cancer potential of fermented and non-fermented Kullakar rice flour porridge, measured through MTT assay. As the concentration increases, the percentage follows an upward trend for anti- cancer inhibition activity of fermented and non-fermented Kullakar rice flour porridge presented in fig 8. The IC_{50} value of standard, fermented Kullakar rice flour porridge and non-fermented Kullakar rice flour porridge is presented in table 3 for anti-cancer inhibition activity. The IC_{50} of standard (Doxorubicin) value was 11.63± 0.87 µg/mL and the IC_{50} value of fermented Kullakar rice flour porridge was 21.27± 1.15 µg/mL. This indicates that the fermented sample required nearly twice the amount to achieve 50% cell death, indicating lower cytotoxicity than the standard (Doxorubicin). Conversely, the non-fermented Kullakar rice flour porridge had a significantly lower IC_{50} value of 5.27± 0.21 µg/mL, showing that it is more potent compared to the standard (Doxorubicin), since it took less than half the concentration to induce the same level of cell death ($p<0.000$).

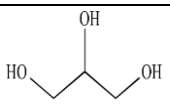
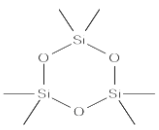
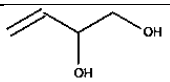
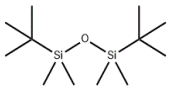
3.5 GC-MS Analysis

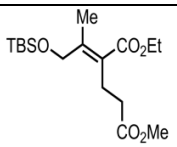
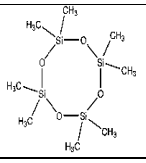
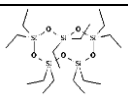
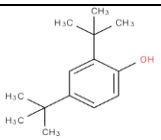
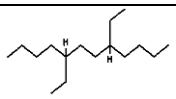
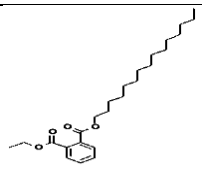
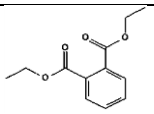
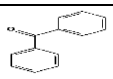
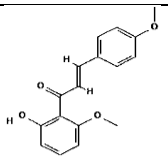
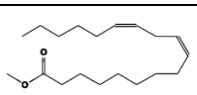
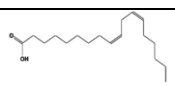
The result of GC-MS analysis of fermented and non-fermented Kullakar rice flour porridge is presented in table 4, fig. 9 and fig. 10. The GC-MS analysis of fermented and non-fermented Kullakar rice flour porridge revealed distinct chemical differences. Common compounds in both samples included hexamethyl-cyclotrisiloxane, dodecane, tetradecane, bis(2-ethylhexyl) phthalate, stigmasterol, and γ-sitosterol. Compounds unique to non-fermented porridge were 3-butene-1,2-diol, n-hexadecanoic acid, eicosane, and cyclobutylamine, while fermentation introduced beneficial compounds like glycerin (100% peak area), 1,2-propanediol, and 9,12-octadecadienoic acid methyl ester. Fermentation increased hexamethyl-cyclotrisiloxane (50.3% to 62.1%) and bis(2-ethylhexyl) phthalate (24.2% to 41.2%) but reduced dodecane (45.3% to 20.0%), n-hexadecanoic acid (59.3% to 20.3%), γ-sitosterol (8.6% to 2.8%), and stigmasterol (2.9% to 1.0%). Additionally, non-fermented porridge contained diethyl phthalate (33.8%) and benzophenone (2.8%), while fermentation introduced phthalic acid ethyl pentadecyl ester (40.9%). These findings highlight fermentation's impact on the porridge's chemical profile, potentially altering its nutritional and functional properties.

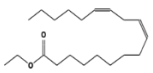
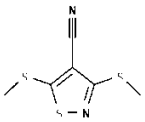
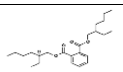
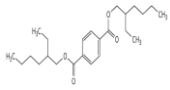
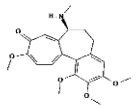
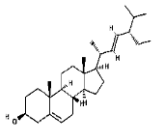
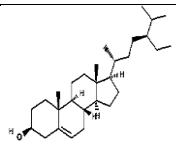
4. Discussion

The qualitative phytochemical analysis of fermented and non-fermented Kullakar rice flour porridge highlights notable differences in bioactive compounds, demonstrating the impact of fermentation on its chemical composition. Both samples contained phytochemicals such as tannins, saponins, flavonoids, terpenoid glycosides, phenolic compounds, and steroids, all of which are recognized for their antioxidant and health-promoting effects [31]. However, a key distinction was observed in alkaloid content—present in the non-fermented porridge but absent in the fermented sample. This suggests that fermentation may facilitate microbial metabolism, leading to the breakdown or transformation of alkaloids and potentially altering the porridge's phytochemical composition [32].

Table 4. Chromatography-Mass Spectrometry Analysis (GC-MS) of Fermented and Non-fermented Kullakar rice flour porridge

Compounds	Structure	RT		Peak Area (%)	
		Fermented	Non-Fermented	Fermented	Non-Fermented
Glycerin		3.5943	-	100.0	-
Cyclotrisiloxane, hexamethyl-		3.9529	3.9857	62.1	50.3
Dodecane		5.0782	5.0945	20.0	45.3
3-Butene-1,2-diol		-	4.3525	-	73.7
Disiloxane, 1,1,3,3-tetramethyl- 1,3-bis[3-(oxiranylmethoxy)propyl]-		6.0881	-	4.9	-

Compounds	Structure	RT		Peak Area (%)	
		Fermented	Non-Fermented	Fermented	Non-Fermented
Hordenin tert-butyldimethylsilyl ether		7.0979	3.4457	4.7	8.5
Cyclobutylamine, N-acetyl-		-	5.3583	-	66.6
Cyclotetrasiloxane, octamethyl-		5.3173	6.4383	9.4	39.2
Tetradecane		7.7698	7.7779	33.3	29.9
Cyclopentasiloxane, decamethyl-		-	9.0145	-	10.4
2,4-Di-tert-butylphenol		9.7937	9.7977	7.4	2.6
1,2-Propanedial, 3-(octadecyloxy)-diacetate		10.4780	-	1.8	-
Dodecane, 5,8-diethyl-		-	10.4819	-	1.2
Phthalic acid, ethyl pentadecyl ester		11.4013	-	40.9	-
Diethyl Phthalate		-	11.4052	-	33.8
Benzophenone		12.1267	12.1266	2.5	2.8
4,6'-Dimethoxy-2'(trimethylsilyl) oxychalcone		-	12.2626	-	2.1
Octadecane		15.3378	15.3376	4.3	3.9
n-Hexadecanoic acid		18.8785	18.8660	59.3	20.3
Eicosane		19.8225	19.8182	5.0	3.5
9,12 Octadecadienoic acid (Z,Z)-, methyl ester		22.6501	-	1.3	-
9,12 Octadecadienoic acid (Z,Z)-		24.0434	24.0515	16.7	22.0

Compounds	Structure	RT		Peak Area (%)	
		Fermented	Non-Fermented	Fermented	Non-Fermented
Linoleic acid ethyl ester		24.7977	-	2.7	-
Isthiazole-4-carbonitrile, 3,5-bis[(2-dimemhylamino) ethylthio]		32.9262	-	2.6	-
Bis(2-ethylhexy) phthalate		34.2739	34.2739	41.2	24.2
1,4-Benzenedicarboxylic acid, bis(2-ethylhexy) esther		36.4381	36.4380	3.5	4.2
Demecolcine		-	38.3341	-	4.7
Stigmasterol		40.6013	40.6012	2.9	1.0
.gamma.-Sitosterol		41.0960	41.1531	8.6	2.8

There was also a notable increase in total phenolic content after fermentation showing enhanced bioavailability due to the breakdown of complex phenolic compounds by microbial enzymes [33]. A slight increase in flavonoid content may be attributed to enzymatic modifications that release bound flavonoids, improving their extractability [34]. Conversely, the reduction in tannin content can be explained by microbial degradation, which reduces antinutritional factors and enhances the porridge's digestibility [35]. Similar observations have been reported in other fermented cereal-based foods, where fermentation enhances the bioavailability of beneficial phytochemicals while diminishing secondary metabolites that may affect taste or bioactivity [36]. These findings underscore the beneficial role of fermentation in refining the phytochemical profile, ultimately contributing to the porridge's enhanced nutritional and functional benefits.

Fermentation plays a crucial role in enhancing the antioxidant potential of Kullakar rice flour porridge as assessed by its free radical scavenging activity like DPPH• radical-scavenging activity and superoxide radical scavenging activity of Kullakar rice flour porridge, as indicated by the significant reduction in IC_{50} values. The fermented porridge exhibits superior antioxidant potential compared to the non-fermented porridge, suggesting the bioconversion of complex polyphenols and flavonoids into more bioavailable forms during microbial fermentation [37]. Although the IC_{50} of fermented porridge remains higher than that of ascorbic acid, it still provides considerable antioxidant activity, reinforcing its potential as a functional food [34]. The improved radical scavenging activity can be attributed to the enzymatic breakdown of bound antioxidant compounds, which enhances their efficacy in neutralizing reactive oxygen species (ROS) [38]. Antioxidant are increasingly recognized for their health benefits, with phenolic and flavonoids compounds serving as key contributors to phytochemical mediated free radical scavenging activity [39, 40]. These findings support the role of fermentation in augmenting the health benefits of traditional rice-based foods by improving their antioxidant properties.

Fermentation has been widely recognized for its ability to enhance the antioxidant potential of cereal-based foods by improving the bioavailability of polyphenols and other reducing compounds. The significant decrease in the IC_{50} value of fermented Kullakar rice flour porridge compared to its non-fermented, suggests that microbial activity during fermentation facilitates the release of bound antioxidant compounds, thereby improving phosphomolybdenum reduction activity [41]. Although the reducing power of the fermented porridge remains lower than that of ascorbic acid, it still demonstrates notable electron-donating capacity, which plays a key role in neutralizing free radicals [42]. Similar findings in other fermented cereal products indicate that fermentation reduces antinutritional factors, such as tannins and phytic acid, which may otherwise limit the bioavailability of antioxidants [43]. The reduction in IC_{50} value after fermentation aligns with previous findings that microbial activity enhances the release of bound antioxidants, improving the functional properties of fermented foods

[44]. These results further support the role of fermentation in boosting the antioxidant efficacy of Kullakar rice flour porridge, making it a superior natural antioxidant source.

Fermentation significantly enhances the protein denaturation inhibition capacity of Kullakar rice flour porridge, as reflected in its lower IC_{50} value compared to the non-fermented counterpart. The fermented porridge demonstrates a protective effect against protein denaturation, bringing its inhibitory potential closer to that of Diclofenac Sodium, a commonly used anti-inflammatory drug. This suggests that fermentation enhances the bioactive compounds responsible for stabilizing protein structures, reducing the likelihood of denaturation [45]. The improved inhibition activity could be attributed to the increased availability of phenolic and flavonoid compounds, which are known to exhibit anti-inflammatory properties by preventing protein denaturation [46]. Flavonoids exhibit anti-inflammatory properties through various mechanisms, including the inhibition of regulatory enzymes and transcription factors that play a crucial role in controlling inflammatory mediation [47,48]. These findings align with studies on fermented plant-based foods, which have demonstrated enhanced anti-inflammatory potential due to microbial transformations that increase bioactive compound efficacy [49]. Thus, fermented Kullakar rice porridge shows promise as a functional food ingredient with potential therapeutic applications.

Fermentation has been shown to enhance the anti-diabetic potential of Kullakar rice flour porridge by improving its alpha-amylase inhibitory activity. The fermented porridge exhibits a lower IC_{50} value compared to its non-fermented counterpart, indicating greater enzyme inhibition. Although the standard inhibitor, Acarbose, remains more potent, the fermented porridge still demonstrates considerable inhibitory effects, suggesting its potential role in regulating postprandial glucose levels [50]. The improvement in enzyme inhibition can be attributed to fermentation-induced biochemical changes, including an increase in bioactive compounds such as phenolics and flavonoids, which have been reported to contribute to anti-diabetic properties [51]. The anti-diabetic activity attributed to bioactive and phenolic compounds, with their composition influenced by soil conditions, climate geographic location and plant age [52,53]. These findings align with previous studies showing that fermentation enhances the functional properties of cereal-based foods, making them beneficial dietary options for glycemic control [54].

The cytotoxicity analysis of fermented and non-fermented Kullakar rice flour porridge, measured by IC_{50} values, highlights a significant reduction in toxicity following fermentation. The fermented porridge exhibited an IC_{50} value of 22.22 µg/mL, indicating that a higher concentration was needed to achieve 50% cell death compared to the standard drug Doxorubicin. This suggests that fermentation reduces cytotoxic effects, making the porridge a safer dietary option [55]. Interestingly, the non-fermented porridge demonstrated a much lower IC_{50} value, indicating a higher potency in inducing cell death, which could be attributed to the presence of certain bioactive compounds that may be altered or diminished during fermentation [56]. The decrease in cytotoxicity post-fermentation aligns with previous studies showing that microbial transformations often lead to detoxification and improved phytochemical profiles in fermented foods [57].

The GC-MS analysis of fermented and non-fermented Kullakar rice flour porridge revealed notable changes in its chemical composition, emphasizing the impact of fermentation on bioactive compound profiles. While both samples contained common compounds such as hexamethyl-cyclotrisiloxane, dodecane, bis(2-ethylhexyl) phthalate, stigmasterol, and γ-sitosterol, fermentation introduced beneficial compounds like glycerin, 1,2-propanediol, and 9,12-octadecadienoic acid methyl ester, which are known for their functional and bioactive properties [34]. Additionally, fermentation significantly increased the concentration of certain compounds, such as hexamethyl-cyclotrisiloxane and bis(2-ethylhexyl) phthalate while reducing others like dodecane, n-hexadecanoic acid, γ-sitosterol, and stigmasterol. The disappearance of diethyl phthalate and benzophenone post-fermentation, coupled with the emergence of phthalic acid ethyl pentadecyl ester, suggests potential detoxification and transformation of certain compounds [58]. These findings align with studies showing that microbial fermentation alters the chemical profile of cereals, potentially enhancing their nutritional and functional properties [59].

The health benefits observed in the results of the present study could be attributed to the bioactive compounds modified by natural fermentation. Glycerin, detected post-fermentation, contributes to antioxidant activity by scavenging free radicals through its hydroxyl groups, which stabilize reactive oxygen species (ROS), as seen in the DPPH and ferric reducing assays [60]. Similarly, 1,2-propanediol (propylene glycol) likely supports antioxidant effects by acting as a free radical scavenger, a property noted in food preservation studies [61]. The linoleic acid derivative, 9,12-octadecadienoic acid methyl ester, plays a significant role in the antidiabetic effects observed in the α-amylase assay. Linoleic acid derivatives inhibit pancreatic α-amylase, slowing carbohydrate digestion and glucose release, which aids glycemic control [62]. This compound also exhibits anti-inflammatory effects by stabilizing proteins against denaturation, as seen in the bovine serum albumin assay, likely by modulating pathways like COX-2 [63].

The anticancer activity against HT-29 cells and anti-inflammatory effects are partly driven by phytosterols like stigmasterol and γ-sitosterol, present in both samples but reduced post-fermentation. These phytosterols inhibit colorectal cancer cell proliferation by inducing apoptosis and cell cycle arrest via pathways like caspase activation, aligning with our MTT assay results [64]. Their reduced levels post-fermentation suggests microbial transformation into more bioavailable forms, potentially enhancing efficacy.

One limitation of the present study is that we did not perform direct microbial enumeration or identification during the natural fermentation process.

Our aim was to closely mimic the traditional home-based preparation of Kullakar rice porridge, where fermentation relies on the indigenous microorganisms present in the rice and environment, without the use of a defined starter culture. This approach ensures that the method remains accessible and reproducible for laypersons in a typical household setting. However, for a deeper scientific understanding, future studies could benefit from assessing and characterizing the specific microbial strains involved in the fermentation process. Such insights would help elucidate the roles of various microorganisms and further enhance the scientific basis of traditional fermentation practices.

5. Conclusion

This study highlights the impact of fermentation on the phytochemical composition, antioxidant potential, and bio-functional properties of Kullakar rice flour porridge. Fermented and non-fermented samples contained key phytochemicals such as phenolics, flavonoids, and saponins, though alkaloids were found only in the non-fermented sample, suggesting their possible degradation or transformation during fermentation. Fermentation significantly increased total phenolic and flavonoid content while reducing tannin levels, thereby improving both bioavailability and digestibility. These shifts in composition not only enhance nutrient absorption but also contribute to the porridge's therapeutic potential. Antioxidant assays revealed enhanced radical-scavenging activity and reducing power in the fermented porridge, reflecting a stronger ability to combat oxidative stress. Additionally, fermentation improved bio-functional properties, with the fermented sample exhibiting more pronounced anti-inflammatory and anti-diabetic activities, likely due to the increased presence of bioactive metabolites. With respect to anti-cancer activity, the non-fermented porridge showed higher cytotoxicity than the fermented porridge. This may be associated with specific compounds that are moderated or neutralized through fermentation. This suggests that fermentation offers a safer alternative with balanced bioactivity, making it more suitable for functional food applications. GC-MS analysis further supported these findings by revealing beneficial biochemical changes and the formation of novel bioactive compounds during fermentation. Overall, fermentation emerges as a natural and effective method to enhance the nutritional, therapeutic, and functional qualities of Kullakar rice flour porridge, underscoring its potential as a valuable addition to health-promoting diets.

Author Contributions: Conceptualization, D.A.B. and J.P.S.; methodology, D.A.B.,J.P.S., R.D.P. and T.H.; validation, A.B.D.; investigation, J.P.S.,T.H. and R.D.P.; data curation, J.P.S.,T.H. and R.D.P.; writing—original draft preparation, J.P.S. and D.A.B.; writing—review and editing, J.P.S. and D.A.B.; visualization, R.D.P and T.H.; supervision, D.A.B.; project administration, J.P.S. and D.A.B.; funding acquisition, J.P.S. and D.A.B. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: We confirm that neither the manuscript nor any parts of its content are currently under consideration or published in another journal. All the authors have approved the manuscript and agree with submission to "Prospects in Pharmaceutical Science"

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