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RESEARCH ARTICLE

Evaluation of Synergistic Effects of Lyophilized Fermented Rice Varieties, *Kattuyanam* and *Mappillai samba*: Phytochemical, Amino Acid, Antinutrient Content, and Bioactive Properties

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Abstract

Background: Traditional rice varieties, *Kattuyanam* (KR) and *Mappillai samba* (MR), are known for their nutritional and medicinal benefits. When combined as KRMR, these rice varieties are believed to exhibit synergistic bioactive properties, including antioxidants, antibacterial, anti-inflammatory, and anticancer effects. **Aim:** This study aimed to evaluate the synergistic bioactive properties of KR and MR rice varieties (KRMR), focusing on their phenol and flavonoid content, amino acid profiles, antinutrient levels, and biological activities, including antioxidant, antibacterial, anti-inflammatory, and anticancer effects. **Methods:** KR and MR rice were combined to form KRMR, and various analyses were conducted. The phenolic and flavonoid content were measured, and amino acid profiling identified key compounds. Antinutrient assays evaluated oxalate, phytate levels, and the inhibition of amylase and glucosidase. Antioxidant activity was assessed using Ferric Reducing Antioxidant Power (FRAP) and DPPH scavenging assays. Antibacterial activity was tested against *Escherichia coli* and *Staphylococcus aureus*, while anti-inflammatory and anticancer effects were tested on HepG2 liver cancer cell lines**. Results:** KRMR showed high levels of phenolic and flavonoid compounds. Amino acid analysis revealed the presence of histidine, threonine, valine, and leucine. Antinutrient assays indicated variable oxalate and phytate levels and inhibition of amylase and glucosidase activities. KRMR demonstrated strong antibacterial activity against *E. coli* and *S. aureus*. Antioxidant assays revealed high FRAP and DPPH scavenging capacities. Additionally, KRMR exhibited significant anti-inflammatory and anticancer activity, particularly against HepG2 cells. **Conclusion:** The combination of *Kattuyanam* and *M. samba* rice (KRMR) enhances bioactive properties, demonstrating strong antioxidant, antibacterial, anti-inflammatory, and anticancer effects. KRMR is a promising source of bioactive compounds with potential health benefits.

Major Findings: The study concludes by highlighting the various nutritional profiles and bioactive characteristics of the *Kattuyanam* and *M. samba* in combination, which suggests its wide variety of health benefits.

Keywords: Antibacterial, Antioxidant, Antiinflammatory, Anticancer, *M. samba*, *Kattuyanam*

1. Introduction

In the world, rice is the primary staple food for more than two-thirds of the people. Approximately 80% of the world's rice production comes from the Asian rice (*Oryza sativa* L.) industry[1]. Rice is consumed as a part of the meal in many countries. However, because of their health benefits, consumption of conventional varieties of rice is expanding. The conventional varieties of pigmented rice varieties are available in reddish, purple, blackish, and crimson colors. The traditional red and black varieties are rich in vitamins and minerals. They have about 30% more protein, and 18% crude fibre[1]. The anthocyanins present in the

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pigmented rice have antioxidant properties. They can prevent the production of free radicals[2].

Fermentation also improves rice's nutritional profile by boosting the number of amino acids, minerals, and vitamins and increasing the products nutritional value, energy content, and medicinal potential[3]. Recently, several health impacts associated with microorganisms that are engaged in the fermentation process have been connected to them, leading to a new area of scientific interest[4]. Research into naturally occurring compounds with antioxidant activity that lessens the deleterious effect was prioritized due to the hazards associated with taking manufactured antioxidants. Fermented foods lower blood cholesterol, minimize the risk of atherosclerotic disease, and prevent cancer[5].

The primary components of proteins and enzymes, which play a vital role in human physiology, are amino acids. Inadequacy of amino acids are linked to a number of illnesses in humans, including immunodeficiency, insomnia, cell destruction, slowing of growth in kids, *etc*., as a result of metabolic disorders[6]. In addition to food nutrients, there are food molecules that are not nutrients but play protective roles in the body. These dietary molecules help food nutrients promote immunity, but their capacity to do so is dependent on their quantities in the system. When levels of phytochemicals in food rise, they may become harmful to the body or function as anti-nutrients[7].

The antioxidant, anti-cholesterol, anti-diabetic and cardio-protective properties were strongly associated with pigmented rice in previous research[8]. *M. samba* rice has anti hypercholesterolemic effect, anticancer activity, potential to increase male fertility, antineurological qualities and antidiabetic effect, have all been documented by numerous investigations[9,10]. The traditional grain *Kattuyanam* was known for its antioxidant capabilities due to its high level of flavonoids and phenolic compounds. It also has a high protein content[11]. In the current study, aminoacid profile, antinutritional content and bioactivity profile of lyophilized Fermented rice *i.e.*, *Kattuyanam* (KR), *M. samba* (MR) and mixed ratio of *M. samba* and *Kattuyanam* (KRMR) were investigated.

2. Materials and Methods

2.1 Sample Collection

Two rice varieties namely, *Kattuyanam* (KR) and *M. samba* (MR) were sourced from a local store in Chennai, Tamilnadu. The Sample code 020122308S- *Oryza Sativa* (var. *M. samba*), 020122309S- *O. sativa* (var. *Kattuyanam*) was authenticated by Siddha Central Research Institute, Chennai and its certificate number is 697.20122308-09.

2.2 Sample Preparation and Optimization

The different samples, *i.e., Kattuyanam*, *M. samba* and *M. samba*: *Kattuyanam* (1:1) were processed according to the parameters given in Table 1. The bacterial growth curve of the samples was analyzed. The biomass was calculated and lyophilized[12].

2.3 Phytochemical Analysis

The qualitative phytochemical screening was performed out using standard protocols[13]. The quantitative analysis for Total Flavonoid Content (TFC) and Total Phenol Content (TPC) were carried out using aluminum colorimetric method and Folin-ciocalteu method respectively[14,15].

Table 1. Optimization of rice varieties

Si. No.	Parameteres	KR	MR	KRMR
1	Sample quantity	30 gm	30gm	15 +15 gm
2	Method	Boiling method	Boiling method	Boiling method
3	Rice: Water ratio (Before cooking)	1:5	1:5	1:5
4	Rice (cooked): Water ratio	1:2	1:2	1:2
5	Cooking duration	30 minutes	50 minutes	55 minutes
6	Fermentation hours	6,8,10,12,16,18, 20,22,24 hours	6,8,10,12,16,18, 20,22,24 hours	6,8,10,12,16,18, 20,22,24 hours

(Note: KR-*Kattuyanam*; MR-*M. samba*; KRMR- *Kattuyanam; M. samba* (1:1))

2.4 Aminoacid Profiling

Amino acid analysis was conducted on silica plates measuring 10x10 cm, using an automated spray-on system. 17 Amino acid standards and three samples were applied with a 5µl syringe. Each 5µl sample formed a 5 mm band with a 10 mm gap between band centres. The plate, loaded with samples and standards, underwent a 20-minute development in a pre-saturated vertical glass chamber. The n-butanol: glacial acetic acid: water (3:1:1 v/v/v) mixture was used as the mobile phase. Amino acids were visualized using ninhydrin reagent, and the plate was then heated at 110° C. The migration distance of the mobile phase was calculated. Post-development, the plates were dried and viewed in a TLC visualizer at different wavelength. The Retention factor (Rf) was calculated.

2.5 Antinutrient Assay

Oxalate content was identified by the titration method described by Day and Underwood[16]. The titration method of Sudarmadji and Markakis was used to estimate the amount of phytate[17]. Using the modified starch iodine technique, the plant samples α-amylase inhibitory activity was assessed[18]. The plant extracts β-glucosidase inhibitory activity was tested using a 96-well plate, as described in a recent study[19]. Using L-dopa as a standard, the ultraviolet m was measured in a spectrophotometer to determine the L-dopa content[20].

2.6 Bioactivity Evaluation

2.6.1 Antibacterial Analysis - Agar Well Diffusion

Cell-free culture supernatants (CFSs) of different *Kattuyanam* and *M. samba* samples were assessed for antibacterial activity using the agar well diffusion assay. Culture supernatants were made by the respective samples in MRS broth overnight at 37C and cells were removed by centrifugation at 2000 g for 10 minutes. *S. aureus* and *E. coli* (food borne pathogens) were diluted in physiological saline (McFarland No. 1) and swabbed on Muller Hinton agar to a final concentration of 3×10^5 CFU/ml. After allowing the agar to solidify, wells of 7 mm diameter were made with a well puncture. CFS was added to wells in various amounts, namely 25, 50, 75, and 100 µl. The plates were left alone for several hours to allow the supernatant to diffuse into the agar. *E. coli* and *S.aureus* were incubated at 37° C for 24 hours before the inhibitory zones were determined[21].

2.6.2 Antioxidant Assay

2.6.2.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay

Using the spectrophotometry approach, the samples ability to scavenge 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radicals was assessed. After dissolving the sample (25 mg) in 3 ml of distilled water, 2.8 ml of the extract solution was combined with 0.2 ml of DPPH methanolic solutions. After letting the solution stand for 30 minutes at room temperature in the dark, the absorbance at 517 nm was measured[22]. The percentage of DPPH radical-scavenging activity was calculated using the following Equation 1.

2.6.2.2 Ferric Reducing Antioxidant Power (FRAP) Assay

Freshly made FRAP reagent (3.0 mL) was preheated at 37° C and combined with 40 µL of sample before being incubated at 37° C. Absorbance at 593 nm was measured in comparison to a reagent blank containing distilled water that was likewise incubated at 37° C for up to 1 hour. The result was expressed as Fe+2/g[23].

2.6.3 Antiinflamatory Assay - Bovine Serum Albumin

Bovine serum albumin was used as a substrate to assess the anti-inflammatory effect[24]. A 0.2% (w/v) BSA stock solution was prepared in a pH 6.8 Tris-acetate buffer. The test compounds were prepared in methanol. 150 µL of methanol and 2850 µl of BSA solution make up the control. The standard drug used was diclofenac. 150 µl of test samples and 2850 µl of BSA solution make up the reaction mixture, which is then incubated for 15 minutes at room temperature. After 4 minutes of heating at 72° C, test tubes were allowed to cool at room temperature for 20 mins and read at 660 nm[25]. The following formula was used to determine the percentage inhibition of denaturation or precipitation of the BSA from the sample solution.

2.6.4 Anticancer - MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide Assay)

HepG2 cell lines were procured fromthe National Centre for Cell Science (NCCS), Pune. HepG2 cell line were seeded at a density of 10,000 cells per well in 96-well plates. The experiment was carried out at varied concentrations of 512-1 µg/ml. MTT was then added to each well at the end of the treatment period and incubated for 2-4 hours at 37° C in 5% CO_2. The colored crystals of formazan produced were then dissolved in 150 µl DMSO. The absorbance was read using a Microplate reader at 570 nm[26].

3. Results

3.1 Optimization of Fermented Sample

The bacterial growth curve of the fermented food product of *Kattuyanam*, *M. samba* was analyzed (Figure 1). The biomass of *Kattuyanam*, *M. samba*, and KRMR were estimated to be 1.38, 1.96 and 2.09 g/L respectively.

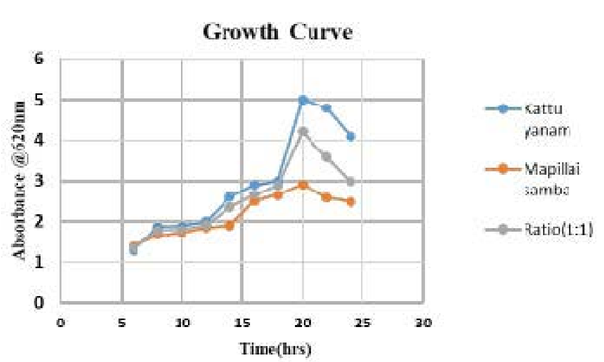

Figure 1. Bacterial growth curve analysis of samples.

Table 2. Qualitative phytochemical analysis

S. No.	Phytochemicals	KR	MR	KRMR
1	Flavonoid	+	+	++
2	Alkaloid	-	-	-
3	Tannin	-	+	+
4	Saponin	-	-	-
5	Terpenoid	+	+	+
6	Phenol	+	+	+
7	Glycoside	-	-	-
8	Steroid	-	-	-

(Note: KR-*Kattuyanam*; MR- *M. samba*; KRMR-*Kattuyanam*: *M. samba* (1:1))

3.2 Phytochemical Analysis

3.2.1 Qualitative Phytochemical Analysis

The presence of different phytochemicals in KR, MR, and KRMR were represented in Table 2. Notably, all three types include Flavonoids, which are known for their antioxidant effects. They lack Alkaloids, Saponins, Steroids, and Glycosides. However, KRMS contains Tannins, which may provide medical benefits.

3.2.2 Quantitative Phytochemical Analysis

3.2.2.1 Determination of Total Phenol Content

The KRMR exhibited the highest Total Phenolic Content (TPC) of 633.71±3.11 mg GAE/ g dry weight. Individually, *Kattuyanam* and *M. samba* exhibited TPC of 301.15±2.78 and 229.23±3.0 mg GAE/100 g dry weight respectively.

3.2.2.2 Determination of Total Flavonoid Content

The KRMR showed the highest flavonoid concentration among the samples, at 952.86±0.84 mg/g. This finding implies that combining the two rice varieties in resulted in a relatively high flavonoid content, indicating the

Table 3. Aminoacid profile of standards

S. No.	Series	Aminoacids	RF Values
1	A1	Aspartic acid	0.206
2	A2	Glutamic acid	0.267
3	A3	Serine	0.194
4	A4	Glycine	0.34
5	A5	Threonine	0.3
6	A6	Arginine	0.077
7	A7	Alanine	0.332
8	A8	Cysteine	0.372
9	A9	Tyrosine	0.457
10	A10	Histidine	0.182
11	A11	Valine	0.498
12	A12	Methionine	0.579
13	A13	Isoleucine	0.555
14	A14	Phenylalanine	0.579
15	A15	Leucine	0.611
16	A16	Lysine	0.182
17	A17	Proline	0.356

potential for enhanced antioxidant properties. In comparison, *M. samba* had a slightly lower flavonoid concentration of 749.90±4.65 mg/g, while *Kattuyanam* had a significantly higher flavonoid content of 573.23±1.78 mg/g[35].

3.3 Aminoacid Profiling

Amino acid profiling of standard and sample was listed in Table 3 and 4. Figure 2 represents aminoacid spots at visible light after spraying. Current study found that five aminoacids were present in *Kattuyanam i.e.,* Leucine, Valine, Threonine, Histidine, Arginine while only 3 aminoacids were identified in *M. samba i.e.,* Alanine, Valine, Leucine. Besides, individual aminoacid profile, four aminoacids were found in KRMR (mixed sample) namely Histidine, Threonine, Valine, Leucine.

Table 4. Aminoacid profile of fermented rice sample (KR, MR, KRMR)

S. No.	Series	Aminoacids	RF Values	Amino Acid
1	A18	*Kattuyanam* (KR)	0.636	Leucine
	A19		0.445	Valine
	A20		0.316	Threonine
	A21		0.17	Histidine
	A22		0.073	Arginine
2	A25	*M. samba* (MR)	0.275	Alanine
	A24		0.478	Valine
	A23		0.606	Leucine
3	A26	*Kattuyanam*: *M. samba* (KRMR)	0.15	Histidine
	A27		0.397	Threonine
	A28		0.416	Valine
	A29		0.673	Leucine

3.4 Antinutrient Analysis

The variations in antinutrient content and inhibitory activities among the samples were evaluated (Table 5). The KR sample exhibits the highest oxalate content at 3.69 mg, which can raise concerns about kidney stone formation due to its binding with calcium. In contrast, KRMR contains the lowest oxalate content at 1.98 mg, making it a preferable choice for individuals prone to kidney stones. Phytate percentages are highest in "KR" at 0.208%, potentially compromising mineral absorption, while KRMR with the lowest phytate content of 0.116% may enhance mineral utilization. KRMR displays better α-amylase inhibition at 372.6 % and significant β-glucosidase inhibition at 132.84 %, suggesting its potential in managing blood sugar and carbohydrate digestion. KRMR showcases the highest L-dopa concentration at 506.57 µg/ml, which can impact neurological and metabolic processes.

3.5 Bioactivity Evaluation

3.5.1 Antibacterial Analysis - Agar Well Diffusion Assay

The inhibitory effects of Cell Free Supernatant (CFS) of KRMR showed better activity compared to individual samples (Table 6). Different concentrations (µl) of CFS against two bacterial strains, *S. aureus* and *E. coli*, in terms of the zone of inhibition (mm) observed around the substance on agar plates. Additionally, the results of using Ciprofloxacin (an antibiotic) with a known concentration of 30µg are included as a reference. Figure 3 (a-f), represents a zone of inhibition (mm) of *Kattuyanam*, *M. samba*, and mixed ratio (KRMR) respectively[41,42].

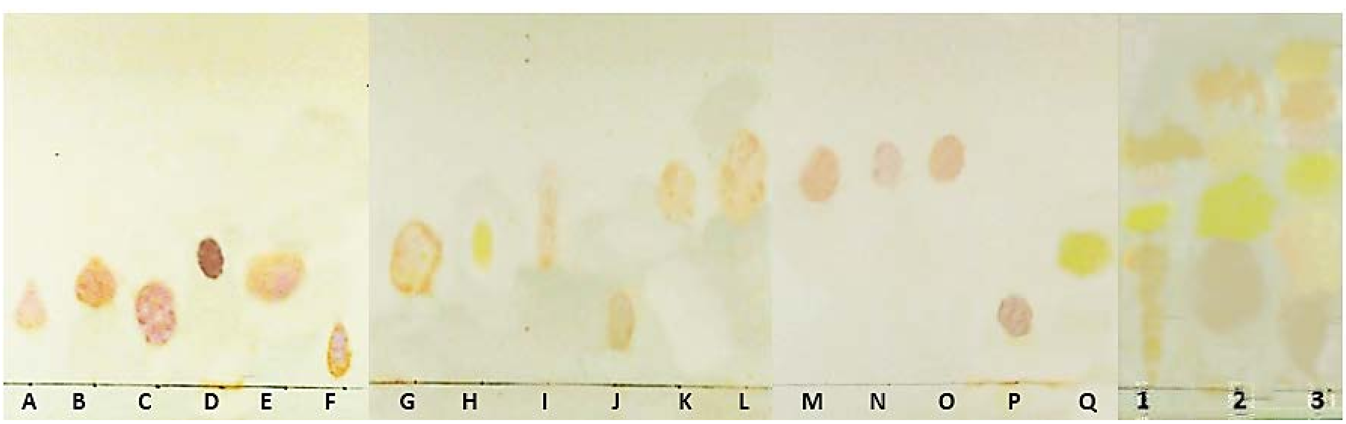

Figure 2. Aminoacid profile- visible light after spraying.

Table 5. Antinutrient analysis of fermented rice varieties

Antinutrient Analysis				
S. No	**Antinutrients**	**KR**	**MR**	**KRMR**
1	Oxalate (mg of oxalate)	3.96±0.22	2.71±0.33	1.68±0.12
2	Phytate (%)	0.232±0.02	0.170±0.03	0.13±0.02
3	α-Amylase inhibitory assay (%)	433.33±1.43	381.91±1.04	372.19±0.42
4	β-Glucosidase inhibitory assay (%)	285.79±0.98	197.79±1.19	143.43±0.77
5	L-Dopa (µg/ml)	446.21±1.80	337.01±0.54	508.75±0.95

Note: KR-*Kattuyanam*; MR- *M. samba*; KRMR- *Kattuyanam*: *M. samba* (1:1)

Table 6. Zone of inhibition of fermented rice varities

Concentration (µL)	KR		MR		KRMR	
	E. coli	***S. aureus***	***E. coli***	***S. aureus***	***E. coli***	***S. aureus***
25	-	-	-	-	-	-
50	-	-	-	-	-	-
75	14.7±0.79	15.36±0.90	13.8±0.81	17.43±0.58	16.43±1.06	18.66±0.94
100	18±0.6	18.83±0.47	17.2±0.62	20.1±0.36	20.6±0.96	23.03±0.65
Ciprofloxacin (30µg/ml)	25.73±0.46	26.06±0.11	26.16±0.28	25.1±0.17	26.06±0.11	25.16±0.28

Note: KR-*Kattuyanam*; MR- *M. samba*; KRMR- *Kattuyanam*: *M. samba* (1:1).

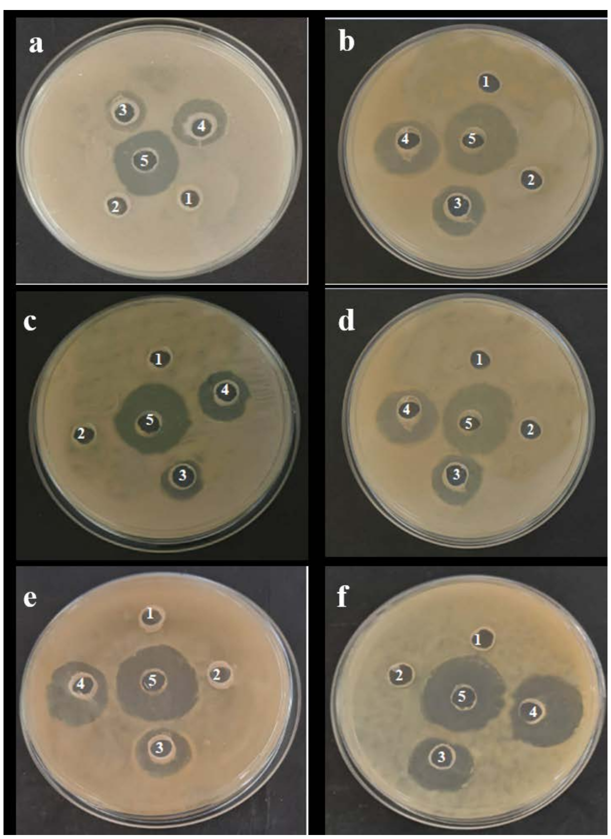

Figure 3. Antibacterial activity (ZOI); **(a)** KR- *E. coli* **(b)** *S. aureus* **(c)** MR- *E. coli* **(d)** *S. aureus* **(e)** KRMR- *E. coli* **(f)** *S. aureus*.

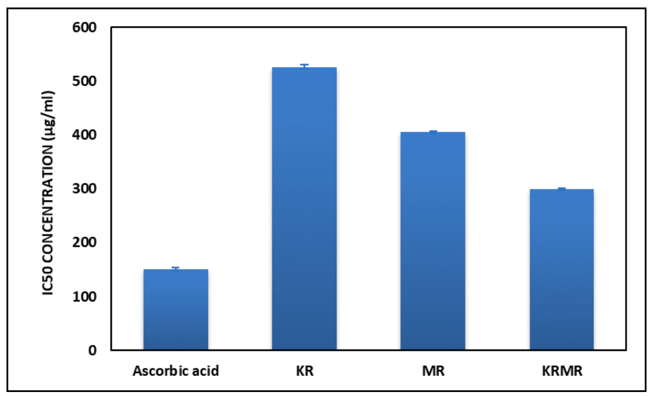

Figure 4. Radical scavenging activity of fermented rice samples (DPPH).

3.5.2 Antioxidant Assay

3.5.2.1 DPPH

The radical scavenging effect of all the three samples increased with an increase in sample concentration (Figure 4). A standard antioxidant, ascorbic acid, has an IC_{50} value of 150.85± 2.49, indicating a considerable amount of inhibitory potential. The IC_{50} value of *Kattuyanam*, was higher at 526.88±3.98, indicating a relatively weaker inhibitory action. While *M. samba*, showed an IC_{50} value of 405.36±2.62.

3.5.2.2 FRAP

The FRAP assay was used to assess the antioxidant capabilities of samples by assessing their ability to reduce ferric ions to ferrous ions. *Kattuyanam*, in particular, has a FRAP value of 284.16±3.65 μg/mg, indicating a significant antioxidant potential. Similarly, *M. samba* had a FRAP value of 420.62±2.72 μg/mg, indicating a strong ferric ion reduction capacity. Moreover, KRMR showed the highest FRAP value measuring 535.83±1.57 μg/mg.

3.5.3 Anti-inflammatory Assay

In the BSA inhibitory assay for anti-inflammatory activity, the results indicate the potential of various substances to inhibit Bovine Serum Albumin (BSA) binding, which is a crucial marker for evaluating anti-inflammatory properties (Figure 5). Diclofenac exhibited a substantial inhibitory effect with a score of 145.59±1.22 μg/ml, showcasing its strong anti-inflammatory capabilities. *Kattuyanam* and *M. samba* exhibited an IC_{50} value of 491.62±1.78 and 360.44±1.27 μg/ml respectively, denoting its potential as an anti-inflammatory agent. Overall, KRMR showed low IC_{50} value of 316.96±2.3μg/ml, suggesting better activity than other two samples.

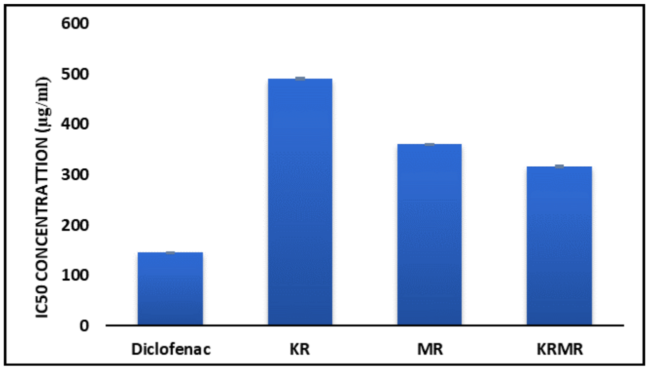

Figure 5. BSA inhibitory assay of fermented rice sample.

3.5.4 Anticancer - MTT Assay

The results of the MTT assay for the compounds *Kattuyanam*, *M. samba*, and mixed sample (1:1) were tested against HepG2 cell lines for various concentrations and were represented in Figure 6. As the concentration increases there is an increase in the cell growth inhibition The IC_{50} values of *Kattuyanam*, *M. samba*, and KRMR were observed to be 146.15±0.42, 115.27±0.53, and 39.14±0.76 μg/ml, respectively

4. Discussion

The optimization of bacterial growth of three rice varieties was observed and it was evident that with increase in time, the bacterial growth increased gradually. Bacterial growth started to decline after the 20th hour. Hence, the optimized condition for both rice varieties was standardized to be 20 hours. The dryweight of KRMR was higher compared to *Kattuyanam* and

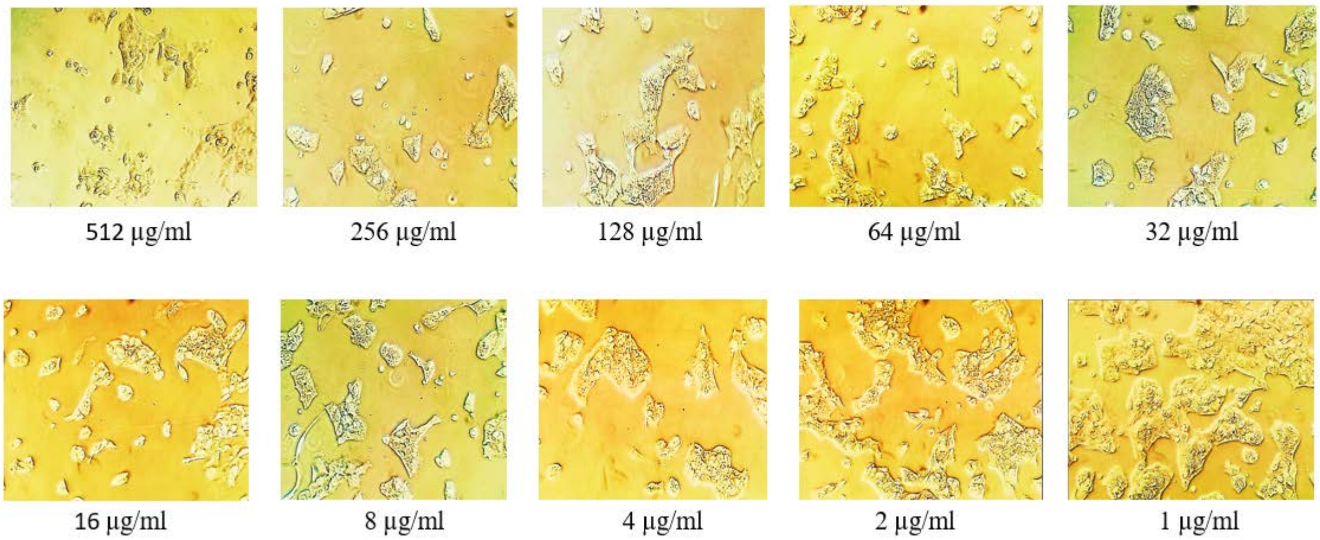

Figure 6. Anticancer activity of KRMR samples against HepG2 cell lines.

M. samba. Brown rice is an excellent source of minerals, antioxidants and bioactive chemicals that are valuable to human health[27]. All three types of optimized freeze-dried rice verities include phenols and terpenoids, both of which have distinct bioactivity. These findings imply that the KRMS retains the presence of essential phytochemicals from its parent types, such as flavonoids, phenols, and terpenoids, making it an ideal source in the diet. Phenol is the most commonly found compound in brown rice. Anti-inflammatory, hypoglycemic, anti-carcinogenic, antiallergenic, and anti-atherosclerotic characteristics are all attributed to phenolic compounds[28,29]. Pigmented rice was proven to have considerably greater quantities of polyphenols, flavonoids, and antioxidant activity than white rice[30]. It has been verified that the quantity of phenols, a phytochemical with one or more hydroxyl groups from aromatic rings, is associated with the antioxidant qualities of grains[31]. Brown rice is richer in phenols than white rice[32]. Previous research revealed that Brown rice has greater antioxidant capacity and higher phenolic content than milled rice[33]. Furthermore, a study conducted by Juan *et al*., suggested that hydrolytic enzymes released by microbes during the fermentation process tend to release the phenolic chemicals that are present plant-based materials[34]. Flavonoids are anti-inflammatory and antioxidant compounds that can inhibit cancer cells proliferation[35]. The phenol, flavonoid, and antioxidant capacities of both pigmented and non-pigmented rice types are strongly correlated with the color parameters[36]. These findings emphasize the potential advantages of blending different rice varieties to produce products with improved nutritional profiles, notably in terms of flavonoid concentration and antioxidant capacity.

Amino acids are often divided into two categories: Essential and non-essential aminoacids. The body is capable of synthesising non-essential amino acids to support ideal development and wellness. Essential amino acids cannot be synthesised in the body and must be obtained through diet[37]. Compared to non-pigmented rice, PR (Pigmented Rice) had greater amounts of EAA (lysine, isoleucine, histone, valine, phenylalanine, methionine and threonine)[38]. The number of amino acids in different foods varies depending on the type of variety, type of soil, genetic background, and other agroclimatic factors[39]. Comparing results with previous study, including pigmented rice samples in diet could provide essential aminoacids required. The health benefits of combined rice sample could be more effective than taking them individually. High soluble oxalate intakes may result in calcium oxalate crystallization and kidney stone development (nephrolithiasis) in the urinary tract which is necessary in analysing antinutrient properties[40]. The capacity of phytate to interact with proteins, carbohydrates and minerals to form insoluble complexes that change the digestion, absorption and functioning of various food constituents has led to the classification of phytate as an antinutrient[41].

The bioactivity results revealed potential disease curing capacity of traditional rice varieties and improved activity by their synergistic effects. The larger the inhibitory zone for the test bacterium, the more bioactive chemicals the extract contains[42]. It was shown in a study using a membrane model that flavonoids, particularly kaempferol, caused damage to the cell membrane in *E. coli*, which may explain why alcoholic extracts of rice bran exhibited stronger antibacterial activity against the bacteria[43]. In addition to antibacterial activity, a previous study revealed that the IC_{50} was low for the rice variety "*Karungkuravai*" (91.08 0.82 g/ml) and high for "*M. samba*" (359.43 24.16 g/ml)[44]. *M. samba* and *Kuruvai kalanjiam* were shown to have considerably higher DPPH scavenging activity than non-pigmented rice extracts. Total phenol concentration, flavonoid content, and DPPH inhibition all showed positive correlations[45]. Overall KRMR, a combination of *Kattuyanam* and *M. samba*, displayed a substantially lower IC_{50} value of 299.85±1.87, indicating a strong inhibitory activity in comparison to the individual rice varieties. Black rice extracts had an antioxidant capacity of 0.06 to 0.63 mg AAE/ml, while red rice extracts had an antioxidant capacity of 0.23 to 1.36 mg AAE/mg[46]. Nonpigmented rice types had FRAP values of 2.02 for husk, 3.05 for bran, 0.87 for whole grain and 0.30 mmol FeSO4/100g for endosperm[47]. Comparing the results of previous studies, it was evident that the antioxidant potential of KRMR showed promising results. Pigmented rice contains a high concentration of medium polar or hydrophilic chemicals such as anthocyanins, phenols, bioflavonoids and proanthicyanidins reduction in inflammation in both *in-vitro* and *in-vivo* models[48]. In the current study, the synergistic effect of MR and KRMR showed efficient reduction of inflammation. The pigmented rice variety *Kattuyanam* of cluster 3 contained p-cresol, which showed anticancer potential[49]. Polyphenols can exhibit several characteristics, such as the potential to reduce cell viability, trigger apoptosis, and regulate inflammation, cell proliferation, tumor formation and metastasis. The bioactive phytochemicals found in the rice bran have been linked to the chemo-preventive potential. The bioactive rice bran components anticancer effects are mediated by apoptotic induction, suppression of changes in cell growth and cell cycle progression in cancerous cells[50]. Overall, in conclusion, the synergistic effect of *Kattuyanam* and *M. samba* showed better effect compared to individual rice varieties.

5. Conclusion

In conclusion, the analysis of *Kattuyanam*, *M. samba*'s fermented samples and their mixture (KRMR) reveals diverse bioactive compounds, amino acid profiles, distinct antinutrient levels, and potential bioactivity. KRMR, combining two different rice samples, emerges as a promising functional food candidate. Amino acid composition highlights essential components crucial for humans, while lower antinutrient content, particularly oxalates, renders KRMR a favorable option for people with kidney stones. Antibacterial assays indicate concentration-dependent inhibitory showcasing KRMR's potential as a natural antibacterial agent. Furthermore, antioxidant assays unveil strong DPPH scavenging and ferric ion reduction capacities. Anti-cancer and anti-inflammatory investigations underscore the potential health benefits of these rice varieties. KRMR exhibits noteworthy inhibitory effects, demonstrating its multifaceted bioactive potential. These findings suggest KRMR as a nutritionally rich bioactive blend, which could be used in the development of food products for health benefits.

6. Acknowledgement

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7. Author Contribution

Conceptualization, R.S. and R.V.; methodology, R.S. and R.V.; validation, RV and VS.; formal analysis, R.S.; investigation, R.V.; resources, R.S.; writing-review and editing, R.S, R.V.; data curation, R.S. and V.S.; writing-original draft preparation, R.S.; and V.S.; visualization, R.S.; supervision, R.V. All authors have read and agreed to the published version of the manuscript.

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