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INITIAL INVESTIGATION ON ANTIOXIDANT ACTIVITY AND TOXICITY OF TRADITIONALLY FERMENTED RICE AND MOISTURIZER FORMULATIONS FOR COSMETIC APPLICATION

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Highlights:

- The initial investigation on rice water from fermented rice which normally used as cosmetic.
- Rice water exhibits potential as a skin moisturizer, but formulation optimization is required.
- Traditional cosmetic product needs to be studied for modern application.

Abstract: "Rice powder," is a Malaysia traditional green skincare remedy historically used for facial care. It's environmentally friendly due to its reliance on rice and water for fermentation, eliminating the need for additional chemicals. The fermentation begins by soaking the rice in water at a 1:1 ratio, with the water refreshed every 14 days over an 84-day period, involving 6 cycles of water changes. The fermented rice powder and soaking water were filtered using a 3-stage filtration steps (muslin cloth, 0.45 and 0.2 µm filter paper) and consequently tested for antioxidants (FRAP, DPPH and TPC) tests. Samples were also tested for toxicity (MTT test) on V79-4 cells. Antioxidant values for rice water were found to be below the standard for cosmetic application, ranging from 1.22, 24.45 and 12.65 for FRAP, DPPH and TPC respectively at concentration 100% (v/v). For fermented rice powder the values are 0.997, 14.6 and 520.15 at 2mg/ml concentration. For 3 moisturizer formulations tested, antioxidant values found to be similar at 0.94, 23.65 and 514.76. Both rice powder and formulations used showed high TPC values. The MTT assay showed that rice water had toxic effects, reducing cell viability from 100% to 4% at concentrations from 1.564 v/v to 50% v/v. In contrast, fermented rice powder showed no toxic effects and even increased cell viability slightly from 100 to 110% as its concentration increased from 0.063 mg/ml to 2 mg/ml. Similar pattern of toxicity were observed by 3 formulations tested indicating its potential for modern cosmetic application.

Keywords: Rice Powder; Rice Water; Green Cosmetic; MTT tests; Antioxidants

1. Introduction

Fermented rice as food products has been known to play an important role in maintaining human health and well-being (Attard et al. 2022; Mishra et al, 2022; Fitri et al, 2025; Nwinyi & Dango, 2025). Rice benefits for cosmetic application are gaining popularity due to consumer concerns about synthetic chemicals/chemical compounds (Kumar 2016; Diaz 2023). Reportedly by Baumann (2024), rice contains many key compounds for cosmetic purposes such as offering antioxidants (fatty, ferulic, cinnamic, protocatechuic acid and vitamin E), strengthen skin barrier (phospholipids, ceramides) and for moisture (ceramides, inositol and oryzanol). Apart from preventing skin aging, the inclusion of antioxidant substances in cosmetics formulation retard oxidation, increase stability and consequently, prolong shelf-life (Ghelichi et al 2023).

In Malaysia, fermented rice has been used for skin care product for ages, locally known as bedak sejuk (cold powder). It is made using traditional methods that have been passed down from generations without any standard methodology. Generally, the rice grains are soaked in water without any addition of chemicals, letting it ferment naturally for months until the rice granules are completely dissolved producing paste powder (Dzulfakar & Tan Kofli, 2015; Dzulfakar et al, 2018). At the end of the process, the rice powder paste is collected and used as skin cosmetic products whereas the soaking water (fermented rice water) is normally discarded.

Apart from the powder paste, the rice water has also reported possess several benefits as beauty care benefits such for hair care especially dandruff (Kumar, 2013). Investigation on rice water even at a day-old rice water contains the bacterium *Bacillus cereus*, which produces the antibiotics zwittermicin A and kanosamine. These antibiotic substances can inhibit the growth of Malassezia furfur, which can cause dandruff. The rice water also reported to be beneficial as skin cares for antiaging/antioxidants (Marto et al, 2018). The water contained 16 amino acids with the highest was glutamic acid, leucine and aspartic acid (Johar et al, 2018), antioxidant (Razak et al, 2017), 17 fatty acids such as linoleic and palmitic (Johar et al, 2018) inositol (Choon-Koo, 2001; Choon-Koo et al, 2004). All these components are common in modern cosmetic ingredients and contribute to soften and brighten the skin (Marto et al, 2018) as well as to cure facial acne and eczema (Joseph et al, 2023). Rice water also hydrates, balances pH levels, and contains ferulic acid, allantoin and antioxidants to combat free radicals and premature aging. Acting as natural toner, it reduces fines lines, wrinkles and promotes collagen for better texture, elasticity and a youthful complexion (Anon, 2024). Ghazali et al (2020) also

reported that the rice water is not cytotoxic to melanoma cells and promotes viability of the cells.

Due to its potential, therefore this study was done to investigate the antioxidant content of the rice water, fermented rice and moisturizer formulation for cosmetic application.

2. Materials and Methods

2.1 Preparation of Fermented Rice Powder

Local rice brand (Jasmine) that contained 5% crushed rice (1 kg) were soaked in tap water at a ratio of 1:1 equal to 1L in a closed container. The rice is allowed to ferment naturally at room temperature. After 14 days, the soaking water is collected and fresh water is added for the fermentation process to continued (Alif et al. 2018). The cycle is repeated 6 times (aprroximately 3 months) for the rice to completely dissolve and turn into a paste.

2.2 Collection of Rice Water

The rice water collected after 14 days fermentation intervals, undergoes a three-stage filtration process. In the first stage, the rice water is filtered using a Muslin cloth filter to separate the rice from the soaking water. Subsequently, the rice water that was filtered with the cloth filter, undergoes further filtration using Whatman filter paper with sizes 0.45 and 0.22 µm continuously (Ibrahim & Kofli, 2019). Sterility test was executed to ensure that the solution is bacteria free. The rice water is then stored in a refrigerator at a temperature of 4°C for further analysis.

2.3 Preparation of Face Moisturizer Formulation

Face moisturizer formulation components used were shown in **Table 1** (control). A total of 20 ml of face moisturizer was prepared, and any formulation that showed physical instability either immediately or after 24 hours of storage at room temperature (25°C) was deemed unsuitable for use as a moisturizer. To create an oil-in-water emulsion cream, the oil phase comprising stearic acid, liquid paraffin, and glyceryl monostearate was heated to 70°C using a magnetic stirrer with a hot plate. The water phase was heated to the same temperature as the oil phase. Both phases were then mixed slowly while continuously stirring with a glass rod to form a homogeneous mixture. Preservatives were added to the water phase before mixing. Borax was used as an emulsifying agent instead of triethanolamine to create a stable formulation (Maru & Lahoti, 2018). The rice water replaced distilled water, and argan oil

replaced lanolin. Lanolin, derived from sheep's sebaceous glands, was substituted with argan oil to ensure the moisturizer remained plant-based.

Table 1. Composition of face moisturizer control formulation

Chemical	Quantity for 100g (%)	Function
	Oil Phase (A)	
Stearic acid	4.0	Emulsifier
Liquid Paraffin	8.0	Occlusive
Argan oil	3.0	Occlusive
Glyceryl monostearate	3.0	Emulsifier
	Aqueous Phase (B)	
Glycerin	4.0	Humectant
Propylene Glycol	4.0	Humectant
Isopropyl myristate	2.0	Emulsifier
Borax	0.2	Emulsifier
Phenoxyethanol	0.1	Preservative
***Distilled water**	71.7	Solvent

*Total volume is 100%

2.4 Cell Culture

V79-4 cells were cultured in tissue culture flasks using DMEM media supplemented with 10% fetal bovine serum, 1mM sodium pyruvate, and 1% antibiotics at 37°C in a humidified atmosphere with 5% carbon dioxide and 95% humidity. The cultures were checked daily to ensure they remained healthy, and any changes in their morphology or adhesion properties were recorded.

2.5 Culture Preparation

To prepare 1 L of DMEM solution, mix DMEM powder with 3.7 g sodium bicarbonate in 900 ml distilled water. Adjust the pH to 7.2-7.4 using 1M hydrochloric acid. Add distilled water to reach 1 L. Filter the solution through a 0.22 µm nitrocellulose membrane filter. Combine 5 mL

Penicillin-Streptomycin and 50 mL 10% FBS with 445 mL of DMEM, making a total volume of 500 ml. Test for contamination by exposing 3-5 mL of the solution in Petri dishes, incubating them at 37°C with 5% CO2 for 72 hours, and examining under a microscope for any contamination.

2.6 Samples Preparation and Formulation

Rice water was diluted to a concentration of 50% v/v in DMEM medium and filtered through a 0.2 µm membrane to sterilize it, as the rice water is not sterile. The sterilized rice water solution was serially diluted to five concentrations (25% v/v, 12.5% v/v, 6.25% v/v, 3.125% v/v, and 1.563% v/v) using DMEM medium. Rice powder was dissolved in DMEM medium at a concentration of 2 mg/ml and filtered through a 0.2 µm membrane to sterilize it. The sterilized rice powder solution was then serially diluted to five concentrations (1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml, and 0.063 mg/ml) using DMEM medium. Face moisturizer formulations (F1, F2, F3: **Table 2**) were dissolved in DMEM medium at a concentration of 2 mg/ml and filtered through a 0.2 µm membrane to sterilize them. The sterilized solutions were then serially diluted to five concentrations (1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml, and 0.063 mg/ml) using DMEM medium.

Table 2. Face moisturizer formulation

	F1 (%)	**F2 (%)**	**F3 (%)**
Stearic acid	4.0	4.0	4.0
Liquid Paraffin	5.0	8.0	10.0
Argan oil	3.0	3.0	3.0
Glyceryl monostearate	3.0	3.0	3.0
Glycerin	4.0	4.0	4.0
Propylene Glycol	4.0	4.0	4.0
Isopropyl myristate	2.0	2.0	2.0
Borax	0.2	0.2	0.2
Phenoxyethanol	0.1	0.1	0.1
***Distilled water**	74.7	73.7	69.7

*Total volume is 100%

2.7 MTT Assay

MTT assay is done to evaluate the cytotoxic potential of the test item (bedak sejuk, rice water, moisturizer formulation). Yellow tetrazolium salt (MTT) is reduced metabolically active cells to form insoluble purple formazan crystals which are solubilized by the addition of a solvent (dimethyl sulfoxide). Cell viability is quantified by colorimetric enumeration whereby a low OD (570nm) reading corresponds to low cell viability (ie associated with a loss in mitochondrial dehydrogenase).

$$Active\ cell\ (\%) = \frac{minimum\ tested\ OD\ cell}{minimum\ negative\ control\ OD} \times 100 \quad (1)$$

2.8 FRAP, DPPH and TPC

All analyses are following standard procedures of these tests. The Ferric Reducing Antioxidant Power (FRAP) assay determined the antioxidant power using ferric reducing ability. A potential antioxidant reduced ferric acid ion (Fe^{3+}) to ferrous ion (Fe^{2+}) which form a blue complex quantified by the increase in absorbance at 593nm. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) measured the capacity of antioxidant to scavenge the DPPH radicals in this assay. A potential antioxidant will react with free radical DPPH causing discoloration of the molecule, which resulted in reduction of the absorbance at 517nm. The total phenolic contents using the Folin-Ciocalteu reagent that oxidise phenolic compound resulted in a blue-coloured reagent which quantified by the increase in absorbance at 765nm.

3. Results and Discussion

3.1 Fermented Rice and Rice Water

Figure 1. shows the pH profile for rice water for 15 days soaking period. Based on the graph, there is a daily decrease in the pH values of the rice water from pH 7 (day 1) to pH 3.8 (day 15).

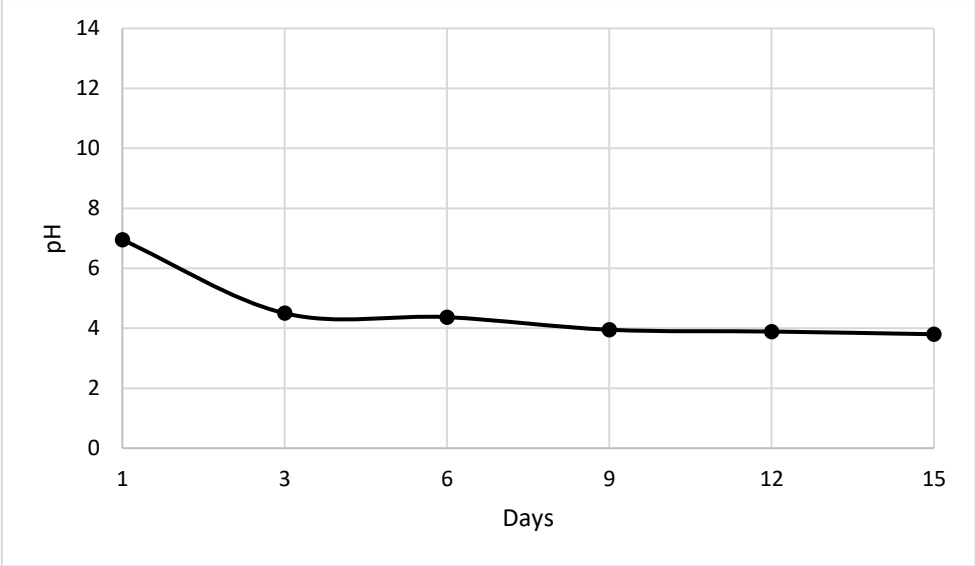

Figure 1. pH profile for rice water during fermentation/soaking

This indicates that the rice water obtained through fermentation is acidic. The drop in pH confirms that natural fermentation of the rice has occurred. This is because the fermentation process is driven by natural microbial activity, which produces lactic acid and acetic acid. Therefore, the decrease in pH is expected when fermentation takes place (Hu et al, 2021; Halim et al 2025).

3.2 Rice Water

Table 3 shows the result for FRAP assay, DPPH assay and TPC assay for rice water. FRAP assay, DPPH assay, and TPC assay are tests used to measure the antioxidant capacity of samples. FRAP assay (Ferric Reducing Antioxidant Power) assesses the antioxidant capacity by evaluating the sample's ability to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). DPPH assay (2,2-diphenyl-1-picrylhydrazyl) measures antioxidant capacity by assessing the sample's ability to reduce the DPPH free radical, which is indicated by a colour change. TPC assay (Total Phenolic Content) measures the total phenolic content in the sample, which correlates with its antioxidant capacity. These three tests are used to evaluate the antioxidant properties of specific samples. Standard benchmarks for FRAP assay are set at 10 mM ascorbic acid equivalent, DPPH assay at 70% radical scavenging capacity, and TPC assay at 5 mg/ml gallic acid equivalent for suitable cosmetic products applications ((Shahidi and Zhong, 2015; Dasgupta et al, 2021). Based on the results obtained, rice water shows limited potential as an antioxidant ingredient for cosmetics due to low activities on all these three indicators.

Table 3. Results for FRAP assay, DPPH assay and TPC assay for rice water

Test	Concentration of rice water	Result	Unit
Ferric Reducing Antioxidant Power (FRAP)	25% v/v	1.023	mM ascorbic acid equivalence
	50% v/v	1.119	
	100% v/v	1.277	
2, 2-Diphenyl-1-picrylhydrazyl (DPPH)	25% v/v	26.9	% radical scavenging capacity
	50% v/v	46.15	
	100% v/v	24.25	
Total Phenolic Content (TPC)	25% v/v	2.41	mg/ml gallic acid equivalence
	50% v/v	3.10	
	100% v/v	12.65	

Based on the MTT assay results obtained from rice soaking water, it shows that rice water has a toxic effect on V79-4 cells. In this test, a test substance is considered toxic if it causes a decrease in cell viability by more than 50% when incubated with cells. According to **Figure 2**, cell viability decreased from 100% to 4% as the concentration of rice water increased from 1.563% v/v to 50% v/v in DMEM medium. However, the exact cause of this reduction in cell viability is not yet identified, whether it is due to toxic components present in the water or the acidic nature of rice soaking water as pH ideals for cell culture growth is at pH7.4 (Well 2023; Li et al, 2022).

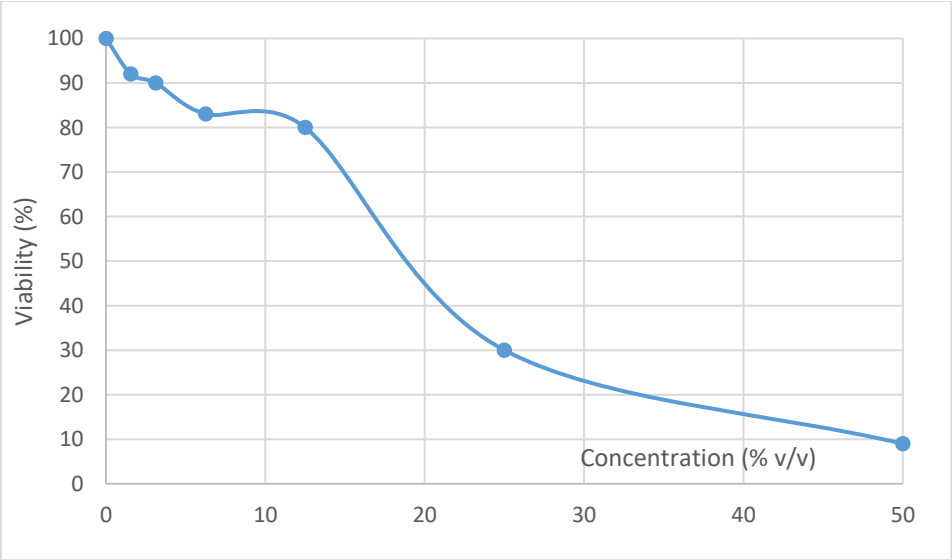

Figure 2. MTT assay results for rice water

3.3 Rice Powder

The antioxidant activity for rice powder is also determined and **Table 4** summarized the data observed.

Table 4. Result for FRAP assay, DPPH assay and TPC assay for rice powder.

Test	Concentration of rice powder	Results	Unit
Ferric Reducing Antioxidant Power (FRAP)	0.5 mg/ml	0.967	mM ascorbic acid equivalence
	1mg/ml	0.940	
	2mg/ml	0.997	
2, 2-Diphenyl-1-picrylhydrazyl (DPPH)	0.5 mg/ml	25.3	% radical scavenging capacity
	1mg/ml	27.9	
	2mg/ml	14.6	
Total Phenolic Content (TPC)	0.5 mg/ml	489.13	mg/ml gallic acid equivalence
	1mg/ml	504.69	
	2mg/ml	520.15	

Based on the results of FRAP assay, DPPH assay, and TPC assay obtained for rice powder, rice powder only meets the standard size for TPC assay as an antioxidant ingredient in cosmetics when it has more than 5 mg/ml gallic acid equivalence at each concentration tested. However, for FRAP assay and DPPH assay, rice powder has less than 10 mM ascorbic acid equivalence and less than 70% radical scavenging capacity, making it unsuitable as a cosmetic ingredient. **Figure 3** shows the result for MTT assay for rice powder.

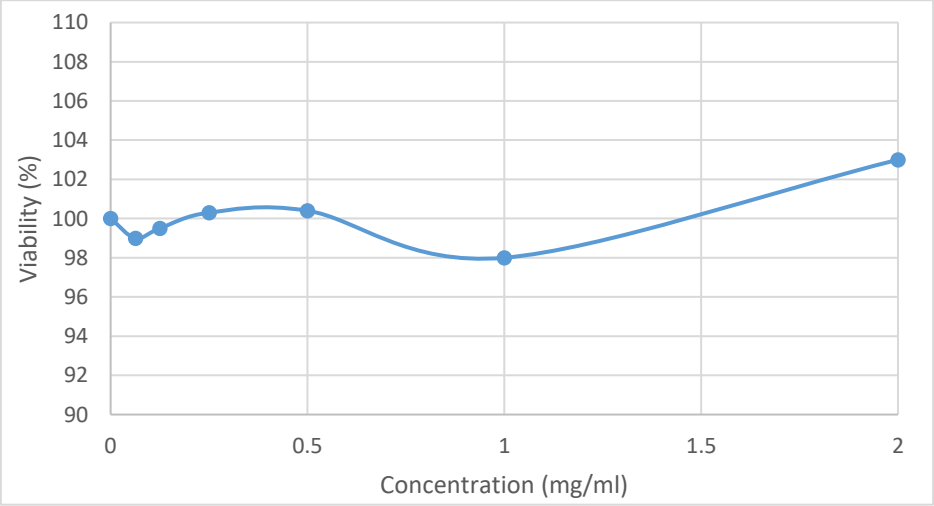

Figure 3. MTT assay result for rice powder

Based on **Figure 3**, rice powder shows no toxic effects on cells. Overall, rice powder maintains cell growth and even shows an increase in cell viability at a concentration of 2 mg/ml, reaching 110%. This proves that rice powder, a cosmetic product that has been around for a long time, truly has a positive effect on human skin. This finding is consistent with previous studies indicating that cold powder is beneficial for treating acne, brightening the skin, and providing a cooling effect.

3.4 Face Moisturizer Formulation

Tables 5, 6 and 7 shows the result for FRAP assay, DPPH assay and TPC assay for all the three face moisturizer formulations (F1, F2 and F3).

Based on the results of FRAP assay, DPPH assay, and TPC assay for face moisturizer formulations (F1, F2, and F3), these three test substances show no significant differences in FRAP assay, DPPH assay, and TPC assay results when the concentration of paraffin is manipulated—specifically, F1 at 5% w/w, F2 at 8% w/w, and F3 at 10% w/w. These test substances also yield results similar to cold powder when only achieving the standard value for TPC assay to act as an antioxidant ingredient, whereas for FRAP assay and DPPH assay, they do not meet the standard value for cosmetic products. Overall, these three face moisturizer formulations show similar results in FRAP assay, DPPH assay, and TPC assay, with FRAP assay results around 0.9 mM ascorbic acid equivalence, DPPH assay at around 24% radical scavenging capacity, and TPC assay at around 500 mg/ml gallic acid equivalence for each concentration.

Table 5. FRAP assay, DPPH assay and TPC assay for F1

Test	Concentration of F1	Results	Unit
Ferric Reducing Antioxidant Power (FRAP)	0.5 mg/ml	0.926	mM ascorbic acid equivalence
	1mg/ml	0.929	
	2mg/ml	0.940	
2, 2-Diphenyl-1-picrylhydrazyl (DPPH)	0.5 mg/ml	29.55	% radical scavenging capacity
	1mg/ml	24.5	
	2mg/ml	24.45	
Total Phenolic Content (TPC)	0.5 mg/ml	500	mg/ml gallic acid equivalence
	1mg/ml	501.64	
	2mg/ml	513.2	

Table 6. FRAP assay, DPPH assay and TPC assay for F2

Test	Concentration of F2	Results	Unit
Ferric Reducing Antioxidant Power (FRAP)	0.5 mg/ml	0.927	mM ascorbic acid equivalence
	1mg/ml	0.929	
	2mg/ml	0.940	
2, 2-Diphenyl-1-picrylhydrazyl (DPPH)	0.5 mg/ml	24.45	% radical scavenging capacity
	1mg/ml	24.19	
	2mg/ml	23.65	
Total Phenolic Content (TPC)	0.5 mg/ml	505.63	mg/ml gallic acid equivalence
	1mg/ml	503.05	
	2mg/ml	514.76	

Table 7. FRAP assay, DPPH assay and TPC assay for F3

Test	Concentration of F2	Results	Unit
Ferric Reducing Antioxidant Power (FRAP)	0.5 mg/ml	0.928	mM ascorbic acid equivalence
	1mg/ml	0.927	
	2mg/ml	0.971	
2, 2-Diphenyl-1-picrylhydrazyl (DPPH)	0.5 mg/ml	23.3	% radical scavenging capacity
	1mg/ml	23.75	
	2mg/ml	21.85	
Total Phenolic Content (TPC)	0.5 mg/ml	502.58	mg/ml gallic acid equivalence
	1mg/ml	501.17	
	2mg/ml	507.97	

Figures 4, 5 and 6 show the result for MTT assay for all the three face moisturizer formulations (F1, F2 and F3).

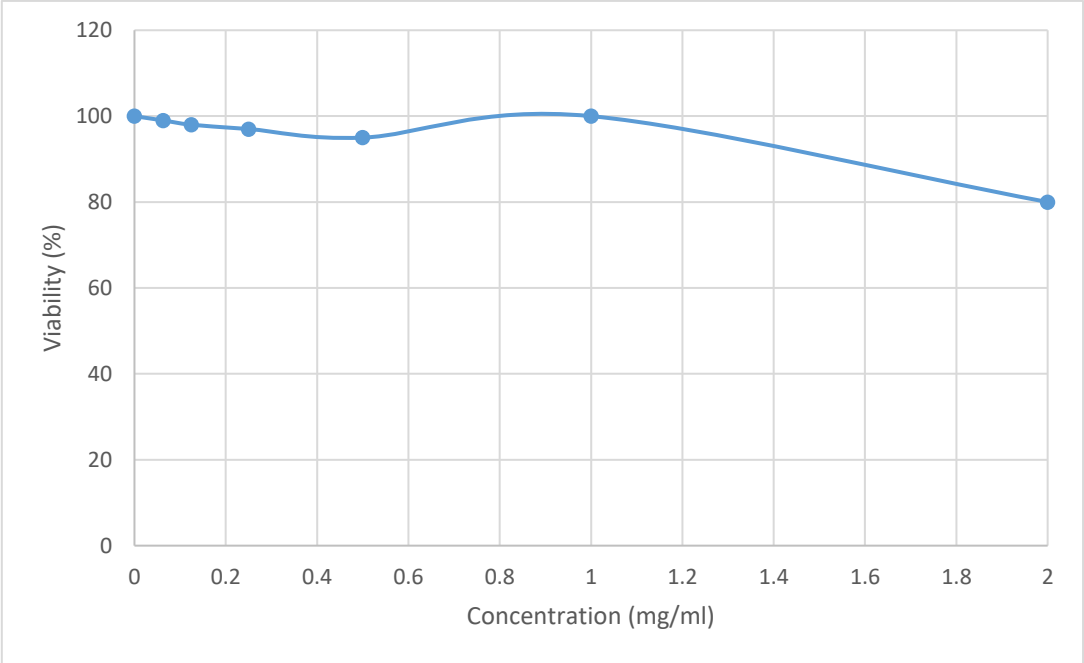

Figure 4. MTT assay result for F1

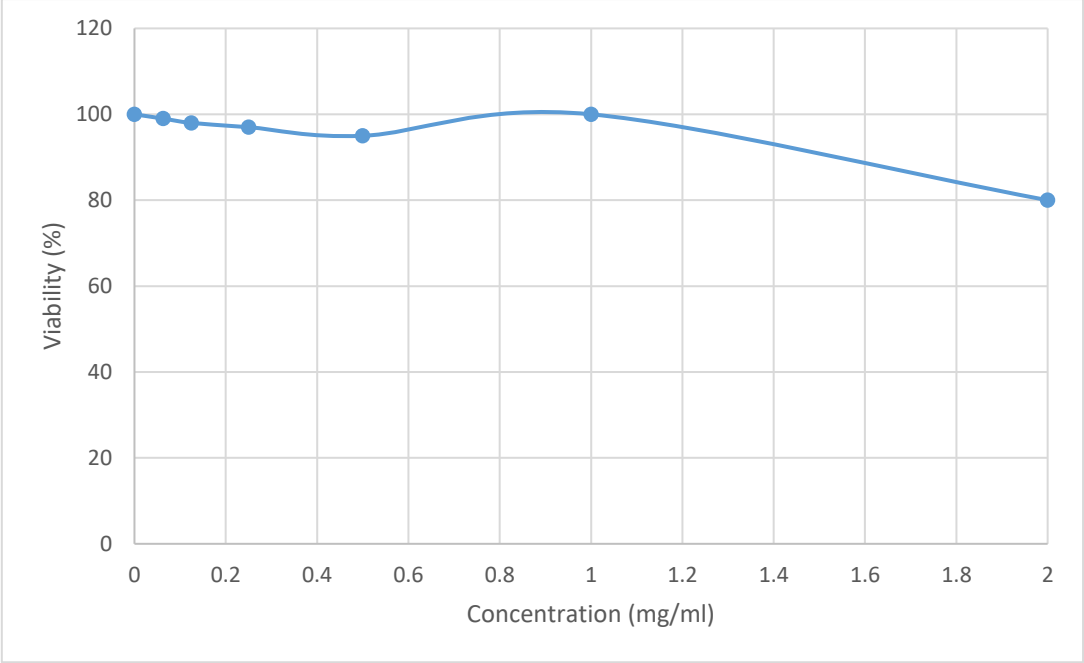

Figure 5. MTT assay result for F2

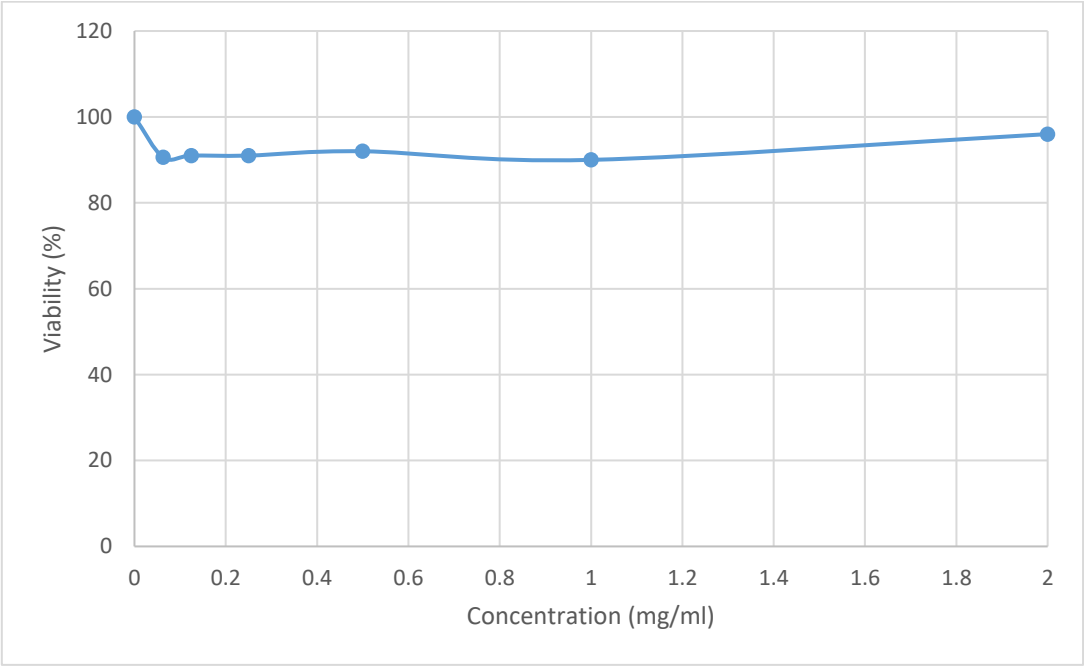

Figure 6. MTT assay result for F3

Based on **Figures** 4, 5, and 6, all three face moisturizer formulations show no toxic effects on cells and maintain cell viability between 80% to 100%. The test substances can sustain cell viability within this range. Face moisturizer formulation F3, which contains 10% w/w paraffin concentration, exhibits the best effect on cells compared to F1 and F2 by maintaining cell viability between 90% to 100%. This demonstrates that a higher concentration of paraffin is

beneficial in cellular contexts because it provides good mechanical stability, prevents water loss and cell dehydration, and is chemically non-reactive, reducing the risk of cell damage. Paraffin also aids in the penetration of certain chemicals into cells and is easily controlled in various biological and medical applications, ensuring the integrity and stability of biological samples during analysis and storage.

4. Limitation and Recommendation

The findings showed the acidic water proved to be harmful to V79-4 cells, making it unsuitable for cosmetic antioxidants. To ensure its useful for cosmetic application, it is suggested that the water be treated to almost neutral pH before application. Improvements on formulation combination need to be statistically tested.

5. Conclusion

In conclusion, the study revealed the collected rice water and rice powder has impacted V79-4 cells. However, cold powder has beneficial effects on skin, meeting antioxidant standards, especially in TPC testing. Tested moisturizer formulations (F1, F2, F3) also meet TPC standards without harming the cells, with F3, containing higher paraffin, showing the best results for mechanical stability and cell safety. These results indicated the potential of bedak sejuk for cosmetic application with better formulation need to be studied.

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Conflicts of Interest

The authors declare no conflict of interest.

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