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Photoprotective potential of black rice extract: LC-MS/MS profiling and cream optimization

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Abstract

Objective

Characterizing the phytochemical profile of black rice (*Oryza sativa* L.) extract using LC-MS/MS, determining and assessing its antioxidant and in vitro photoprotective activities, and formulating an optimized oil-in-water cream containing the extract as a natural photoprotective agent were the aims of this study.

Materials and methods

The black rice grains of North Toraja, Indonesia, were first macerated with a hydroethanolic solvent and then further characterized using LC-MS/MS. This technology is expected to resolve the major phytochemical constituents of the extract. Antioxidant activity, total phenolic and flavonoid contents, and photoprotective activities were determined using the DPPH spectrophotometry and UV-Vis spectrophotometry, respectively, at various concentrations of the extract. A cream base was optimized using Simplex Lattice Design with stearic acid, triethanolamine (TEA), and glycerin as the independent variables, targeting appropriate ranges of pH and viscosity. The optimized base was then loaded with 2.5, 5, and 10% of the extract and the resulting creams were evaluated for physicochemical properties, stability, and SPF.

Results

The total amount of dry extract yielding 11.34% and total phenolic and flavonoid were 15.68 ± 2.12 mg GAE/g and 24.56 ± 0.69 mg QE/g, respectively. The extract was proven to have strong activity as an antioxidant and exhibited activity in a concentration-dependent manner on photoprotection where the SPF values increased from 6.90 to 15.5 and %Te and %Tp decreased at varying concentrations. A complex composition of extracts obtainable by LC-MS/MS including several polyphenols and lipophilic amines/amides was revealed. From the simplex Lattice Design, in a formulation comprising stearic acid 10%, 3% TEA, and 13.5% glycerin, having pH ≈ 6.1, viscosity ≈ 16,000 cPs were achieved and found to be in good agreement with the model predictions. The formulation of the creams with an extract concentration of 2.5 to 10 % was found to be stable and retained pH 4.5 to 4.6, viscosity, and homogeneity as well as the type of emulsion in O/W in the order of 5.7 SPF to 7.1 at 10% extract for the baseline at 10%, usable organoleptic properties after conducting stability tests.

Conclusion

The antioxidant and in vitro photoprotective activities of Black rice extract and its stability in O/W cream base formulation SPFs was enhanced. The combination of phytochemicals LC-MS/MS and formulation optimization streams the approach for black rice photoprotective products and as a multifunctional active ingredient in natural sunscreen products.

Keywords: antioxidant activity, black rice (*Oryza sativa* L.), cream formulation optimization, LC-MS/MS, photoprotective activity

Paper Type: Research Paper.

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Introduction

Data from various studies shows that black rice (*Oryza sativa* L.) has increased interest from the scientific community, as the pigmented cereal has a wealth of nutrients, especially bioactive compounds such as, anthocyanins, flavonoids, and phenols. Phenols and flavonoids are positively correlated to the antioxidant properties of black rice, so it is no surprise that black rice outperformed rivals, like white and red rice, in antioxidant properties (Xie et al., 2020; Zheng et al., 2020). On the other hand, oxidative stress has a known correlation to the development of chronic metabolic disorders, as well as, skin damage from ultraviolet (UV) radiation, and the aforementioned bioactive compounds help to relieve the oxidative stress (Guo et al., 2022; Tai et al., 2020). Cumulatively, these results show that black rice is more than a functional food, as it has also gained recognition for being a natural source of bioactive compounds for nutraceutical and cosmeceutical products (Dhankhar and Kaur, 2023). Unfortunately, irrespective of the possible benefits, black rice is often underutilized in pre-prepared and functionally validated topical formulations. Research has primarily attributed the antioxidant qualities of black rice to the anthocyanins and flavonoids (most especially to cyanidin-3-glucoside) due to its hydrogen donation and electron transfer mechanisms (Banerjee et al., 2023). The recent study illustrated a significant and direct relationship, correlating the total phenolic content (TPC) and total flavonoid content (TFC) to the DPPH assay's free radical scavenging activity (Tyagi et al., 2022). Differing accounts clarify the extent to which the phenolic black rice compounds can be moderately heated and the effect on their antioxidant potential, showing that it would still be sustainable for the intended formulations (Bhawamai et al., 2016; Suryanti et al., 2020). That being said, most studies focused on the presence of antioxidant activity, instead of linking it to a more intricate phytochemical analysis of the extracts. In addition to antioxidants, compounds within black rice have the potential to protect against the skin damaging effects of the sun as well. UV-A and UV-B radiation and the formation of UV-induced reactive oxygen species are known to be absorbed and reduced by use of flavonoids and anthocyanins (Lefahal et al., 2018; Lekmine et al., 2021). The use of UV spectrophotometry to quantify the sun protection factor (SPF) of selected phenolic-rich plants and plant-derived photoprotective extracts, while not the gold standard, has become usual practice to establish the probable photoprotection of these plants (Vijayakumar et al., 2020; Yang et al., 2017). Many researchers have claimed that phenolic-rich plant extracts result in low-to moderate SPF and should be considered as added photoprotection in herbal formulations associated with sunscreen (Fernandes et al., 2023; Mejía-Giraldo, 2021). The phenolic and flavonoid composition of black rice and its photoprotective potential have yet to be documented. Phytochemical characterization involving the use of liquid chromatography-tandem mass spectrometry (LC-MS/MS) is an important method in assessing bioactive compounds found in complex mixtures such as pigmented rice (Vogeser and Seger, 2010). However, some anthocyanin-rich extracts undergo LC-MS/MS analysis, and there are certain challenges with respect to the matrix suppression effect. In such cases, one must be cautious with compounds and try to correlate their identification with certain quantitative markers such as TPC, TFC, and some of the metabolic activities (Liu et al., 2015). In addition, the formulation of cosmeceutical products must also be done in an orderly manner with regards to the plan to ensure there is enough of the desired physical and chemical stability, as well as the working functionalities of the end dosage form. Among some of the methodology reported to address this are the Simplex Lattice Design (SLD) employed, which has been reported to optimize the cream formulations by assessing the interactions of various members of the key components such as the emulsifier and humectants (Kurnianingrum and Zulkarnain, 2023; Saryanti and Prameswari, 2024). For the reasons presented, this paper aimed to evaluate the yield from extraction and the phytochemical elements of black rice extracts and the antioxidative and in vitro photoprotective activities, to bioactively assess black rice extracts from the complex mixtures and cream formulations, and to employ Simplex Lattice Design to optimize cream formulations. Such an approach is sought to further legitimize the use of black rice in nutraceutical and cosmeceutical formulations.

Materials and methods

Sample preparation: In this study, black rice (*Oryza sativa* L.) was obtained from the Rantepao District of North Toraja Regency in South Sulawesi, Indonesia. The rice was washed several times with running water before dried in an oven at 40°C. The dried grains were blended into a fine powder, which was then sieved with a 16 mesh sieve. The powder was kept in sealed containers before the extraction process (Yoshimura et al., 2012).

Extraction process: One thousand grams of black rice powder was transferred into a maceration vessel, to which a solvent at the ratio of 1:3 (solid to solvent) was added. The extraction solvent was an 70% ethanol and 3% citric acid solution at a ratio of 85:15. The solution was left to macerate for 3 days with stirring at regular intervals. The mixture was then filtered, and the extraction process was repeated three times with the same solvent composition. The combined filtrate was evaporated to obtain the remaining dry extract, which was subsequently stored after weighing for future use (Sari et al., 2021).

LC-MS/MS analysis: To profile the chemicals in the three extracts, an ACQUITY UPLC® H-Class system (Waters, USA) connected to a Xevo G2-S QTof mass spectrometer (Waters, USA) was used. The chromatography was performed using mobile phase A (water + 5 mM ammonium formate) and mobile phase B (acetonitrile + 0.05% formic acid) at a flow rate of 0.2 mL min^{-1} using a 23-minute step-gradient. An injection volume of 5 µL was used (all samples were pre-filtered with 0.20 µm syringe filters). Ionization was done in positive mode using electrospray. Data were acquired and processed using MassLynx v4.1 (Waters). Possible identification was done by analyzing the accurate mass, isotopic patterns, and specific diagnostic MS/MS fragments (Khairi et al., 2025).

Antioxidant activity assay: A variation of the DPPH radical scavenging method was utilized to test the antioxidant potential of the black rice extract. 500-2500 µg/mL stock solutions were made by taking 250, 500, 750, 1000, and 1250 µL of the sample stock solution and diluting it to 5 mL with 70% ethanol, to which 1 mL of 0.4 mM DPPH was added, and the mixture was pipetted and incubated for 30 minutes at room temperature. A UV-Vis Spectrophotometer was used to read the absorbance of the solutions diluted with ethanol to 515.5 nm. All readings were done in triplicates. A blank sample was prepared which contained only ethanol and DPPH and the percentage of inhibition was calculated according to formula (Pérez et al., 2018)

$$\% \text{ Inhibition} = \frac{\text{A control} - \text{A sampel}}{\text{A control}}\ x\ 100$$

Regression analysis was conducted to assess the IC_{50} value by plotting extract concentration against the DPPH inhibition percentage.

Total flavonoid content: For the preparation of the solution, 10 mg of the extract was blended in 10mL ethanol and its concentration level was adjusted to 1000 ppm. In 2mL of the solution, 0.1mL 10% $AlCl_3$ and 0.1mL sodium acetate of 1M concentration was added. The solution was made to 5mL ethanol and was then homogenized, after which the sample was let sit for 30 min to incubate. The absorbance at 431 nm was measured to assess the concentration of the sample and the same method was used to demonstrate (Nur et al., 2023).

Total phenolic content: We also measured the total phenolic content. 10 mg of the extract was blended in ethanol to create 1000 ppm solution and was measured. To 0.5 mL of the solution, 2 mL of sodium carbonate was added, and 0.4 mL of Folin-Ciocalteu reagent of sodium as well. The solution was made to 5 mL water and was then homogenized and the same method was used to demonstrate (Nur et al., 2023).

In vitro sunblock assay: To prepare the stock solution of the extract at a concentration of 1000 µg/mL, 50 mg of the sample extract was weighed and then dissolved using absolute ethanol (p.a.). Subsequently, the solution was brought up to a final volume of 50 mL using the same ethanol. For the working solution concentrations of 100-500 µg/mL, 1-5 mL of the stock solution was diluted to the 5 mL mark with ethanol. UV-Vis absorbance spectra were obtained in the 292.5 - 372.5 nm range using a microplate reader. The weighted average spectral transmittance was used to calculate erythema (%Te) and pigmentation (%Tp) according to (Nurlila et al., 2024):

$\%\ Te = \sum T \times Fe \sum Fe$; $\%\ Tp = \sum T \times Fp \sum Fp$

The spectrophotometric Sun Protection Factor (SPF) was calculated as

$$\text{SPF}\ spectrophotometric = \text{CF} \times \sum EE \times I \times Abs$$

where λ 290 to 320 nm and CF = Correction Factor. EE(λ) = Erythemal effect spectra, I(λ) = Solar intensity spectra, A(λ) = Sample absorbance.

Optimization of cream base using simplex lattice design (SLD): The cream base formula was optimized using Simplex Lattice Design with three independent variables, (i) stearic acid (5-15%), (ii) triethanolamine (0.2-0.4%), and (iii) glycerin (12-15%). The variables used to determine response surface were viscosity and pH. The range of formulation variables was based on preliminary optimization (Table 1 and 2). The formulation also contained (iv) cetyl alcohol

(0.5%), (v) phenoxyethanol (1%), (vi) DMDM hydantoin (0.1%), (vii) oleum rosae (qs), and (viii) distilled water to 50 mL. As per Table 1 Base Cream Formula Optimization Design is formulated on the basis of the following percentages of components listed in the Table 3.

Cream formulation: The cream is prepared by first preparing an oil-in-water (O/W) base. This base is made of two different pairs, i.e., oil phase and aqueous phase. For the oil phase, stearic acid, cetyl alcohol and phenoxyethanol are weighed and placed in a porcelain dish, and then melted in a water bath at 80 degrees Celsius. For aqueous phase, triethanolamine, propylene glycol, DMDM hydantoin, glycerin, and water are poured in a beaker and heated to 80 degrees Celsius. Once melted, the oil phase is slowly integrated with the water phase, and the mixture is homogenized constantly until the cream base is formed (Khairi et al., 2018). Subsequently black rice extract (*Oryza sativa* L.) is added to the cream base and homogenized to properly dispersed the active ingredient. Finally, a few drops of oleum rosae were incorporated, and a range of homogenization is conducted until the cream is smooth and homogeneous.

Table 1. Base cream formula optimization design

Ingredient	Concentration (%)
Stearic acid	5-15
Triethanolamine	0.2-0.4
Glyserin	12-15
Cetyl alcohol	0.5
Phenoxyethanol	1
DMDM hydantoin	0.1
Oleum rosae	qs
Purified water	ad 100

Results and discussion

The black rice powder after being macerated with an acidified hydroethanolic solvent system showed an 11.34% recovery yield (Table 4). Similar findings can be found in the literature with the usage of acidified ethanol solvent systems for the enhancement of polyphenolic extractions and the stabilisation of anthocyanins within pigmented rice matrices with extraction yield percentages ranging between 8 to 15% (Dhankhar and Kaur, 2023; Zheng et al., 2020). Citric acid was stabilising anthocyanins at an acidic pH that would trigger the formation of the flavylium cation and improve the stability during extraction. The TPC in the sample was 15.68 ± 2.12 mg GAE/g extract and TFC was 24.56 ± 0.69 mg QE/g extract (Table 4). Black rice, due to its unique flavonoids, anthocyanins, and phenolic acid makeup, has been reported to have a significantly higher phenolic and flavonoid profile than its rice counterparts, notably red and white rice (Banerjee et al., 2023; Huang and Lai, 2016; Xie et al., 2020). This relatively high TFC suggests that the flavonoids were a substantial portion of the phenolic compounds, and continuing the predominant findings of the flavonoids, this added to the antioxidant potential of the pigmented rice (Devraj et al., 2019; Tyagi et al., 2022).

Table 2. Experimental design matrix generated by design expert

Run	Factor		
	Stearic acid (%)	TEA (%)	Glycerin (%)
1	5	0.2	15
2	15	0.2	12
3	15	0.2	12
4	15	0.4	12
5	5	0.4	12
6	5	0.2	12
7	5	0.4	12
8	15	0.4	12
9	15	0.2	12
10	15	0.2	15
11	15	0.4	15
12	5	0.4	12
13	15	0.2	15
14	15	0.4	15
15	5	0.2	15
16	15	0.4	12
17	15	0.4	15
18	5	0.4	15
19	5	0.2	12
20	5	0.2	12
21	5	0.2	15
22	5	0.4	15
23	15	0.2	15
24	5	0.4	15

Table 3. Cream formulation containing black rice extract

Ingredient	Function	Formula (%w/v)			Range (%)
		F1	F2	F3	
Black rice extract	Active ingredient	2.5	5	10	-
Stearic acid	Emulsifier	10	10	10	1-20
Triethanolamine	Alkalizing agent	3	3	3	2-4
Cetyl alcohol	Thickening agent	4	4	4	2-5
Glycerin	Emolient	13.5	13.5	13.5	≤30
Propilenglykol	Humectant	5	5	5	≤15
Dmdm hydantoin	Preservative	0.1	0.1	0.1	0.1-1
Phenoxyethanol	Preservative	1	1	1	0.5-1
Oleum rosae	Fragrance	qs	qs	qs	-
Purified water	Solvent	ad 100	ad 100	ad 100	-

Table 4. Result %yield, antioxidant, TPC and TFC of black rice extract

Sample	Extraction yield (%)	IC_{50} Antioxidant (µg/mL)	TPC (mg GAE/g of extract)	TFC (mg QE/g extract)
Black rice extract	11.34	36.73	15.68 ± 2.12	24.56 ± 0.69
Quersetine		2.31		

Based on the DPPH assay conducted on the black rice extracts, an IC_{50} value of 36.73 µg/mL was obtained, indicating high antioxidant activity, as shown in Table 4. This result demonstrates activity at the same level, or at least as high as, a number of other plant extracts, and not far from the activity of some significant antioxidants such as quercetin. This result further supports the antioxidants of black rice polyphenols (Banerjee et al., 2023; Pérez et al., 2018). As illustrated in the trend of the reducing power in Figure 1, the radical scavenging activity was concentration dependent. This was explained by the strong correlation with TPC and TFC values. This aligns with several researchers who established a significant correlation at a p level, or a level of probability less than 0.05, with antioxidant activity. Similarly, it was established in black rice (Sriseadka et al., 2012; Xie et al., 2020; Yao et al., 2010). This result was primarily attributed to the antioxidant cyanidin-3-glucoside anthocyanin, which donates hydrogen atoms to the oxidative medium while stabilising a molecule of free radical such as superoxide (Tai et al., 2020; Zheng et al., 2020). In previous research, it was shown that black rice phenolics have high bioactivity after extraction and low to medium degrees of thermal processing.

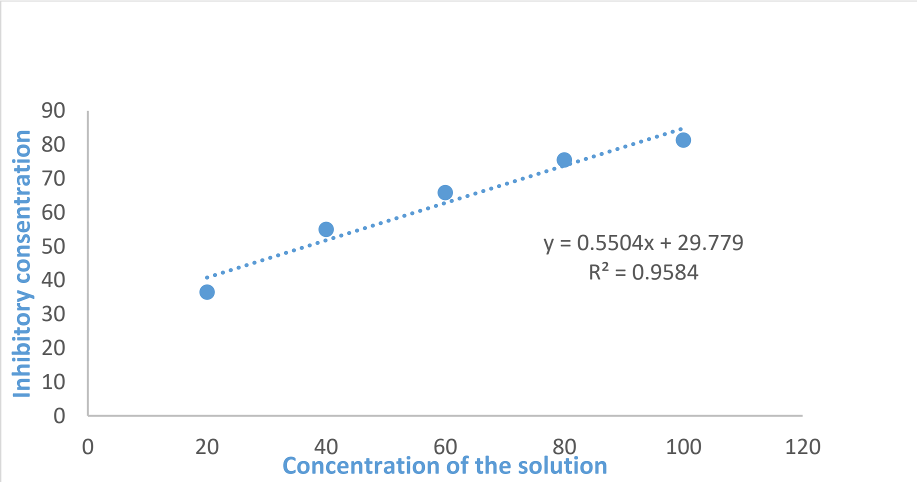

Figure 1. Graph representing the reducing power of the extract of black rice against DPPH

LC-MS/MS profiling identified various bioactive compounds in the black rice extract (Figure 2 and Table 5). Among these, quercetin-3-O-glycoside stood out due to its well-documented antioxidant, anti-inflammatory, and photoprotective properties (Banerjee et al., 2023). Additionally, the detection of linolenic acid and monoacylglycerols suggests the presence of lipid-derived components that may support skin barrier function and impart emollient properties in topical formulations. The compound identification was based on accurate mass measurements and MS/MS fragmentation patterns, which conform to established best practices for phytochemical analysis in complex matrices such as pigmented grains (Vogeser and Seger, 2010; Wang et al., 2014). The absence of dominant anthocyanins, such as cyanidin-3-glucoside, may be attributed to limitations in ionization mode or matrix suppression effects—common challenges in LC-MS/MS analysis of highly pigmented extracts (Liu et al., 2015; Schulz-Trieglaff et al., 2008).

Nevertheless, the presence of flavonoid glycosides aligns with the TFC results and substantiates the observed antioxidant activity.

Table 5. Identified phytocompounds from LC.MS/MS analysis

No	Retention time (min)	Observed MS (m/z)	Molecular Ion	Compound name	Molecular formula
1	3.868	449.10	[M+H]+	Quercetin-3-O-glycosidase	$C_{28}H_{47}N_3O_6$
2	11.497	279.23	[M+H]+	Linolenic acid	$C_{18}H_{30}O_2$
3	12.460	520.33	[M+H]+	Pleiocarpinene	$C_{22}H_{28}N_2O_2$
4	13.00	454.23	[M+H]+	1-acyl-sn-glycero-3-phoshocholines	$C_{21}H_{44}NO_7P$
5	15.623	353.26	[M+H]+	1-monoacylgycerols	$C_{19}H_{38}O_4$

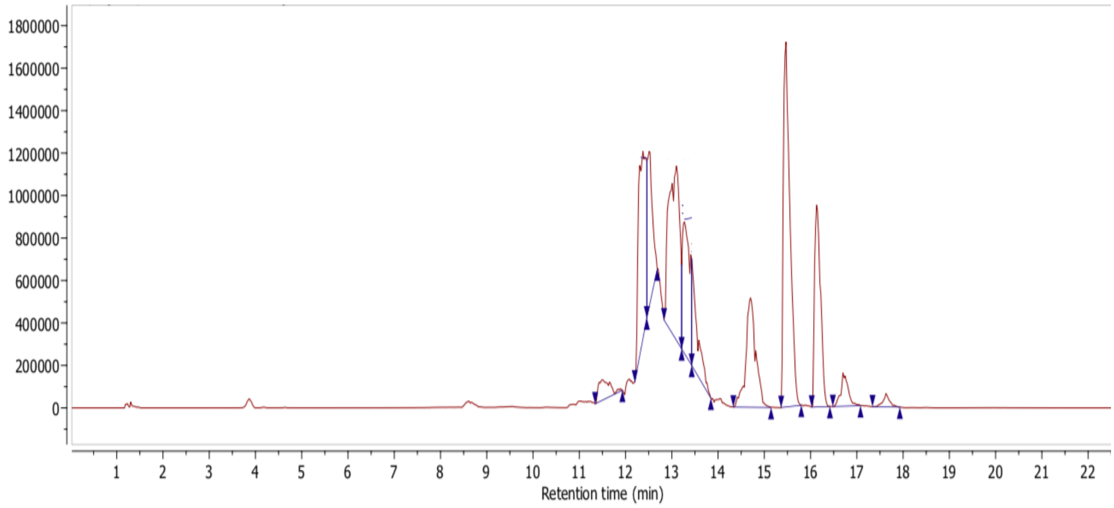

Figure 2. LC/MS.MS profile

The spectrophotometric examination of the sun protection parameters showed that black rice extract has the potential to be photoprotective, as illustrated in Table 6, where an increase in the extract concentration from 100 to 1000 ppm consistently decreased the erythema transmission (%Te) and pigmentation transmission (%Tp) with an increase in the SPF from 6.9 to 15.5. Such values were deemed low and moderate protection and were in line with other phenolic-rich plants' extracts with similar in vitro studied methodologies (Mejía-Giraldo et al., 2016; Nurlila et al., 2024; Vijayakumar et al., 2020). The photoprotective effect is related to polyphenols, more specifically flavonoids and anthocyanins, which absorb UV-A, UV-B, and UV-induced oxidative stress (Lefahal et al., 2018; Lekmine et al., 2021). This property, alongside the other, as antioxidants, may be the reason why there is more support to the claims of the extracts from pigmented grains for being multifunctional active components in sunscreen compositions (Fernandes et al., 2019; Yang et al., 2017).

Table 6. %Te, % Tp and SPF value of Black rice extract

Consentration (ppm)	%Te	%Tp	Categori	SPF	SPF Category
100	0.19	0.50	Sunblok	6.90	Low protection
300	0.13	0.41	Sunblok	8.40	Low protection
500	0.07	0.31	Sunblok	11.10	Low protection
1000	0.02	0.18	Sunblok	15.5	Medium protection

The 24 experimental runs, which were produced by the SLD, were dispersed across the design space of the three excipients and provided formulations with a pH between 6.19 and 7.68 and a viscosity between 3000 and 49,000 cps (Table 7). Some of the runs showed pH levels out of the physiological range of maximum topical preparations (4.5-6.5) demonstrating the significance of balancing the concentration of TEA to prevent extreme alkali levels. Simultaneously, extremely high viscosities like run 23 might be detrimental to spreadability and user comfort, and extremely low viscosities like run 1, 12, 15 and 21 may be linked to insufficient consistency and instability. These results are consistent with earlier research indicating that both the content of the fatty alcohols co-emulsifier and the level of emulsifier influence the viscoelastic characteristics and the size of the droplet in O/W creams (Suena et al., 2024). Design Expert® software indicated a single optimum composition of 10% stearic acid, 3% TEA, and 13.5% glycerin, with a desirability index of 0.387 and responses pH 6.8 and viscosity 16,291 cPs (Table 8). The predicted pH is a bit more than the initially set upper limit, but balances with TEA-neutralized systems pH=6.8. The pH still falls at the acceptable ranges for the wider spectrum of pH values for formulations of cosmetic creams. Experimental confirmation resulted in pH 6.12 and viscosity 16,000 cPs with the one-sample t test analysis indicating no significant difference ($p > 0.05$ for both responses; Table 9) between predicted and observed values indicating the polynomial model sufficiently and properly described response surface for the design space in consideration. The level of confirmation between the experiments and predictions is comparable with the optimization of plant-based sunscreens using a response surface and factorial methodology to pre-formulate and select the parameters of the other ingredients based on the envisaged targets (Putri et al., 2018; Sjahjadi et al., 2021; Wisudyaningsih, et al., 2023). The best base was then used to make three creams with amounts of black rice extract in them. These creams had 2.5, 5 and 10 percent black rice extract. They were called FI, FII and FIII. We looked at what these creams were like. Compared them to a cream with no black rice extract in it. When we looked at the creams, we saw that they were a lilac to dark purple color and they smelled like roses. This is because of the black rice extract that was in them. All of the creams were the type with oil drops in water, before and after we tested them to see if they would last. We put the results in Table 10. Black rice extract was what made the creams smell good and look nice. The batches and conditions all had the consistency. This shows that adding the extract did not hurt the way the emulsifying system worked. We expected the color to change a little over time because the system has a lot of anthocyanin. This change, in color might just be the chromophores moving around a bit. Does not mean the functional activity is getting worse. This is what happens with creams that have a lot of color like the ones that (Keshavarzi et al., 2021; Sari et al., 2021). The anthocyanin is what makes the color change. The emulsifying system is still working well with the extract added. The pH values of FI-FIII were between 4.50 and 4.64 at first and between 4.50 and 4.59 after they were stable for a while (Table 10).

Table 7. Evaluation results for pH and viscosity responses

Run	Factor			Responses	
	Stearic acid (%)	TEA (%)	Glyserin (%)	pH	Viscosity(cps)
1	5	0.2	15	7.53	3000
2	15	0.2	12	6.3	26000
3	15	0.2	12	6.22	23000
4	15	0.4	12	6.28	17000
5	5	0.4	12	7.6	4000
6	5	0.2	12	7.21	9000
7	5	0.4	12	7.57	5000
8	15	0.4	12	6.31	18000
9	15	0.2	12	6.19	24000
10	15	0.2	15	6.2	41000
11	15	0.4	15	6.42	27000
12	5	0.4	12	7.68	3000
13	15	0.2	15	6.25	33000
14	15	0.4	15	6.37	25000
15	5	0.2	15	7.24	3000
16	15	0.4	12	6.33	21000
17	15	0.4	15	6.35	28000
18	5	0.4	15	7.65	3000
19	5	0.2	12	7.1	9000
20	5	0.2	12	7.5	7000
21	5	0.2	15	7.3	3000
22	5	0.4	15	7.4	6000
23	15	0.2	15	6.28	49000
24	5	0.4	15	7.56	4000

Table 8. Optimal composition suggested by Design Expert

Ingredient	Concentration (%)	Function
Black rice extract	2.5-10	Active ingredient
Stearic acid	10	Emulsifier
Triethanolamine	3	Alkalizing agent
Glycerin	13.5	Humectant
Cetyl alcohol	0.5	Co-emulsifier
Phenoxyethanol	1	Preservative
DMDM hydantoin	0.1	Preservative
Oleum rosae	qs	Fragrance
Aquadest	ad 100	Vehicle

This is a range for things you put on your skin like creams and lotions and it is close to the natural pH of your skin. The pH values of FI-FIII went down a bit after they were stored for a while. This might be because the phenolic groups in the values of FI-FIII started to break down slowly or because of small reactions with water. The pH values of FI-FIII are not the ones that do this. It happens a lot in mixtures that have acids and parts of plants, in them like the ones that (Purwanto et al., 2022; Wisudyaningsih et al., 2023).

Table 9. One Sample T-test results comparing predicted formula with experimental optimum and statistical significance

Response	Predicted Value	Experimental Value	Desirability	Significance (p-value)
pH	6.8	6.12	0.837	0.082
Viskositas (cps)	16.291	16000	0.837	0.326

The viscosity values of the semisolid creams stayed within a range and only went down a little bit after we tested them for a while. This means that the creams did not really change or break down. This is what we expected to happen because glycerin and cetyl alcohol help keep the cream strong and able to hold water. The glycerin and cetyl alcohol make the cream matrix stronger on the inside (Kohri et al., 2011; Wadkute et al., 2025). The glycerin and cetyl alcohol are important, for the semisolid creams. The adhesion and spreadability results also fell within the specified limits, indicating that the optimized base maintained a favorable balance between firmness and ease of application, an aspect critical for consumer acceptability (Baptista and Freitas, 2022).

Table 10. Evaluation of cream formulations before and after stability testing

Parameter	Before Formula			After Formula		
	I	II	III	I	II	III
Organoleptic						
- Colour	LC	PP	DP	LC	PP	DP
- Aroma	RS	RS	RS	RS	RS	RS
- Form	SL	SL	SL	SL	SL	SL
Homogeneity	HG	HG	HG	HG	HG	HG
pH	4.55 ± 0.06	4.62 ± 0.07	4.64 ± 0.04	4.50 ± 0.10	4.51 ± 0.10	4.59 ± 0.06
Viscosity (cPs)	7.23± 0.21	7.80 ± 0.25	8.76 ± 0.35	6.73 ± 0.15	7.23 ± 0.40	8.06 ± 0.68
Adhesion (s)	2.24± 0.21	2.49± 0.06	2.74± 0.28	2.17± 0.15	2.49± 0.05	2.68± 0.30
Spreadabillity (cm)	6.32± 0.16	5.50± 0.15	65.42± 0.25	6.46± 0.15	5.85± 0.10	5.74± 0.20
Emulsion type	O/W	O/W	O/W	O/W	O/W	O/W

Notes: LC = Lilac; PP = Purple; DP = Dark Purple; RS = Rose; HG = Homogeneus; SL = Semi-liquid; O/W = Oin in water emulsion

The SPF of the final formulations of the cream (Table 11) indicated a small increase over the base cream, at 6.2 to 7.1, resulting values are in the category of "low protection". The values, although much lower than that of the synthetic sunscreens, are comparable with other herbal cream formulations. These values also serve to justify the incorporation of black rice extract as a substantive photoprotective agent and not as a standalone sunscreen (Baldisserotto et al., 2018; Ragunath et al., 2025).

Table 11. %Te, % Tp and SPF value of Black rice extract cream formulations

Sample	%Te	%Tp	Category	SPF	SPF Category
Base	0.25	0.57	-	5.7	-
FI (2.5%)	0.22	0,55	Sunblok	6.2	Low protection
FII (5%)	0.22	0,55	Sunblok	6.3	Low protection
FIII (10%)	0.19	0,49	Sunblok	7.1	Low protection

Several deficiencies still remain however which must be acknowledged. Due to the lack of authentic reference standards, the structural confirmation was limited as the identification of the compound using LC-MS/MS was only tentative. Also, the photocontrol assessment was only using in vitro spectrophotometry, which may not correspondingly reflect the true performance in vivo on the human integument. On the other hand, the variation of anthocyanin content on the other hand which derives from the differences of cultivar, growing or cultivation altitude, and post-harvest handling was not considered, albeit these elements are recognized to influence the phytochemistry of black rice greatly (Dhankhar & Kaur, 2023). Further research using standardised cultivars, in vivo SPF measurements, and long-term stability studies are needed to improve the translational potential of black rice extract in nutraceutical and cosmeceutical.

Conclusion: The extract of black rice (*Oryza Sativa* L.) is exhibited to have numerous varieties of phenolic and flavonoid compounds and shown to have antioxidant and quantifiable photoprotective properties. Acidified hydroethanolic is a good extraction technique to obtain the highest total phenolic and flavonoid content extract that is closely related to the DPPH radical scavenging activity of the extract. Its content of bioactive flavonoid glycoside and lipid-derived components that potentially interact relatedly to enhance antioxidation and skin barrier to render the extract more effective also agrees with other works. The photoprotective potential of the extract is potentially useful in supportive protective ability in sunscreen as it gives the extract the ability of reducing UV induced erythema and pigmentation. Formulation researches revealed that black rice extract cream formulation systems can be optimized using the Simplex Lattice Design. The recipes maintained stability with the right amounts of pH, viscosity and sensory properties. All of the protective values of the developed black rice extract containing, creams were comparable with other herbal formulations and support the idea that the extract can be used as a complementary to a sunscreen ingredient instead of an active one.

Author contributions

NK: Conceptualization, Writing - Original draft preparation. MN: Methodology, Softwere Data curation. KH and FN: Visualization, Investigation. MI: Supervision and Writing - Reviewing and Editing. MR: Validation.

Data availability statement

The information that backs up what we found in this study is in the article and the extra things that come with it. If you want to see the information from the tests we did and other sets of data, we got during this study you can ask the main author for it and they will give it to you if you have a good reason, for wanting it.

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Ethical considerations

This study did not have any people or animals in it. So, the people in charge did not need to give their okay. All the things we did in the lab were done the way following the rules for being safe and honest, in our research.

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Conflict of interest

The authors declare no conflict of interest.

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پتانسیل محافظت نوری عصاره برنج سیاه: پروفایل‌سازی LC-MS/MS و بهینه‌سازی کرم

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چکیده

هدف: هدف از این مطالعه، شناسایی پروفایل فیتوشیمیایی عصاره برنج سیاه (Oryza sativa L.) با استفاده از روش LC-MS/MS، تعیین و ارزیابی فعالیت آنتی‌اکسیدانی و فعالیت محافظت نوری در شرایط in vitro، و فرمولاسیون و بهینه‌سازی یک کرم امولسیون روغن در آب حاوی عصاره برنج سیاه به‌عنوان یک عامل طبیعی محافظت‌کننده در برابر نور بود.

مواد و روش‌ها: دانه‌های برنج سیاه جمع‌آوری‌شده از منطقه نورث توراجا در اندونزی ابتدا با حلال هیدرواتانولی به روش ماسراسیون استخراج شدند و سپس با استفاده از تکنیک LC-MS/MS مورد شناسایی ترکیبات قرار گرفتند. این روش به‌منظور شناسایی ترکیبات اصلی فیتوشیمیایی عصاره به کار گرفته شد. فعالیت آنتی‌اکسیدانی، میزان کل ترکیبات فنولی و فلاونوئیدی، و فعالیت محافظت نوری عصاره در غلظت‌های مختلف با استفاده از روش DPPH و اسپکتروفوتومتری UV–Vis ارزیابی گردید.

پایه کرم با استفاده از طراحی آماری Simplex Lattice Design و با در نظر گرفتن اسید استئاریک، تری‌اتانول‌آمین (TEA) و گلیسرین به‌عنوان متغیرهای مستقل و با هدف دستیابی به محدوده مناسب pH و ویسکوزیته بهینه‌سازی شد. سپس پایه بهینه با غلظت‌های ۲/۵، ۵ و ۱۰ درصد عصاره غنی‌سازی شد و کرم‌های حاصل از نظر خواص فیزیکوشیمیایی، پایداری و فاکتور محافظت در برابر آفتاب (SPF) مورد ارزیابی قرار گرفتند.

نتایج: بازده عصاره خشک برابر با ۱۱/۳۴٪ به‌دست آمد و میزان کل فنول‌ها و فلاونوئیدها به‌ترتیب ۲/۱۲ ± ۱۵/۶۸ میلی‌گرم معادل اسید گالیک بر گرم عصاره (mg GAE/g) و ۰/۶۹± ۲۴/۵۶ میلی‌گرم معادل کوئرستین بر گرم عصاره (mg QE/g) بود. عصاره فعالیت آنتی‌اکسیدانی قوی نشان داد و فعالیت محافظت نوری آن به‌صورت وابسته به غلظت افزایش یافت، به‌طوری که مقادیر SPF از ۶/۹ به ۱۵/۵ افزایش یافت و درصد عبور پرتوهای UV (Te% و Tp%) با افزایش غلظت کاهش پیدا کرد. آنالیز LC-MS/MS وجود ترکیبات پیچیده‌ای شامل پلی‌فنول‌ها و آمین‌ها/آمیدهای لیپوفیل را نشان داد. بر اساس طراحی Simplex Lattice، فرمولاسیونی شامل ۱۰٪ اسید استئاریک، ۳٪ TEA و ۱۳/۵٪ گلیسرین با pH حدود ۶/۱ و ویسکوزیته حدود ۱۶۰۰۰ سانتی‌پواز به‌عنوان فرمولاسیون بهینه شناسایی شد. کرم‌های حاوی ۲/۵ تا ۱۰٪ عصاره از نظر pH (۴/۵ –۴/۶)، ویسکوزیته، یکنواختی و نوع امولسیون روغن در آب پایدار بودند و SPF آن‌ها تا ۷/۱ در غلظت ۱۰٪ عصاره افزایش یافت. همچنین ویژگی‌های ارگانولپتیک قابل قبولی پس از آزمون‌های پایداری حفظ شد.

نتیجه‌گیری: عصاره برنج سیاه دارای فعالیت آنتی‌اکسیدانی و محافظت نوری in vitro قابل توجهی است و افزودن آن به فرمولاسیون کرم روغن در آب موجب بهبود SPF و پایداری فرمولاسیون می‌شود. ترکیب شناسایی فیتوشیمیایی با LC-MS/MS و بهینه‌سازی فرمولاسیون، رویکردی مؤثر برای توسعه محصولات محافظت نوری مبتنی بر برنج سیاه و استفاده از آن به‌عنوان یک ماده فعال چندمنظوره در ضدآفتاب‌های طبیعی فراهم می‌کند.

کلیدواژه‌ها: برنج سیاه (Oryza sativa L.)، بهینه‌سازی فرمولاسیون کرم، فعالیت آنتی‌اکسیدانی، LC-MS/MS، فعالیت محافظت نوری

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