



Investigation of Bioactive Compounds, Free Radical Scavenging Activities, Cytotoxicity, and Anti-Inflammatory Properties of Thai RD43 Rice Extracts for Use as Novel Cosmetic Ingredients

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Abstract: This study investigated the bioactive compounds, antioxidant, anti-inflammatory, and cytotoxic activities of ethanolic extracts from white and brown RD43 rice, and evaluated the stability of a topical emulsion formulation containing the selected extract. Extraction with 70% ethanol by maceration yielded a higher extract percentage from brown rice than from white rice. Both extracts contained phenolic acids, flavonoids, and γ -oryzanol; however, the brown rice extract exhibited significantly higher levels of total phenolics, flavonoids, γ -oryzanol, and phenolic acids. Consequently, the brown rice extract showed superior antioxidant activity, with DPPH radical scavenging activity ($IC_{50}=1.90\pm0.05$ mg/mL), ferric reducing antioxidant power (374.12 ± 2.75 mg TE/g of extract), hydroxyl radical scavenging activity (223.77 ± 3.27 mg TE/g of extract), and superoxide radical scavenging activity (176.42 ± 6.01 mg TE/g of extract). Cytotoxicity evaluation demonstrated that both extracts were non-toxic to normal human dermal fibroblast (NHDF) cells. Furthermore, the brown rice extract significantly inhibited nitric oxide production in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages ($p<0.05$), indicating anti-inflammatory activity. Due to its superior biological activities, the brown rice extract was incorporated into a cream formulation, which exhibited acceptable physicochemical characteristics and preliminary stability during storage. These findings suggest that RD43 brown rice extract is a promising natural source of bioactive compounds with antioxidant and anti-inflammatory properties and has potential for application as a functional ingredient in cosmetic formulations aimed at protecting the skin against oxidative stress and inflammation.

Keywords: Thai RD43 rice extract; Free radical scavenging activities; Anti-inflammatory activity; Skin care cream

1. Introduction

The use of alternative natural components in skincare products has gained popularity in recent years because of their potential to reduce or prevent skin issues such as hyperpigmentation and aging. This is accomplished by either reducing inflammation brought on by UV exposure (UV-induced inflammation) or mitigating the effects of free radicals produced by UV radiation (UV-induced

reactive oxygen species, ROS). The skin's antioxidant surveillance system combines a synergistic network of low-molecular-weight compounds (like ubiquinol and GSH) and enzymes (like glutathione peroxidase and SOD) to neutralize reactive oxygen species (ROS). A breakdown in this homeostatic regulation leads to oxidative stress, characterized by oxidative modification of genomic DNA, structural proteins, and phospholipid membranes [1-2]. Moreover, the immediate cutaneous inflammation—specifically, erythema and swelling—observed in healthy tissue after sun exposure is caused by UV-induced ROS acting as secondary messengers that start pro-inflammatory cascades [3]. These oxidative processes are primarily driven by singlet oxygen ($^1O_2$), the hydroxyl radical (OH), hydrogen peroxide (H_2O_2), and the superoxide radical anion ($O_2^{\cdot-}$) [4]. Ultraviolet B (UVB, 290–320 nm) radiation promotes cytotoxicity in dermal fibroblasts and accelerates ROS generation within cells, according to a prior study [5]. These reactive intermediates trigger complex pro-inflammatory signaling cascades that can result in dyspigmentation, accelerated photoaging of the dermal matrix, and rhytids (wrinkles). Remarkably, treatment with resveratrol-enriched rice (RR) reduced these negative effects. Furthermore, oxidative stress can raise reactive oxygen species levels and trigger the release of pro-inflammatory cytokines such as TNF- α and IL-6, as well as inflammatory mediators such as nitric oxide (NO). Aberrant nitric oxide (NO) production is closely associated with chronic inflammatory states, which may precipitate genotoxicity and neurotoxicity. Consequently, the pharmacological inhibition of NO biosynthesis is recognized as a pivotal strategy for mitigating the progression of inflammation-mediated pathologies [6].

Because of their strong antioxidant properties, the strategic integration of botanical secondary metabolites into specialized industries has grown dramatically [7]. According to empirical data [8], extracts rich in phenolic and flavonoid compounds are efficient redox homeostasis modulators that can reduce UV-induced oxidative stress. Additionally, their capacity to inhibit pro-inflammatory signaling pathways emphasizes their usefulness as multipurpose components in contemporary nutraceutical and dermo-cosmetic applications. Of these, rice extract is particularly noteworthy as a natural component commonly used in cosmetic formulas. A variety of secondary metabolites with strong radical-scavenging abilities make up the phytochemical matrix of non-pigmented *Oryza sativa* L. Ferulic, sinapic, and p-coumaric acids are important phenolic monomers that frequently occur with flavonoid glycosides and flavan-3-ol polymers (proanthocyanidins) like catechin. Importantly, the aleurone layer of these types is the main source of tocopherols and gamma-oryzanol, which add to the bran extract's overall antioxidant activity. These molecules are known to have numerous biological functions, including antioxidative actions [9]. According to earlier research, Thai rice crude extracts show significant free radical scavenging properties and high amounts of phenolic and flavonoid components [10]. According to Teeranachaideekul et al. [11], Luem-Pua (LP), a kind of Thai pigmented rice, exhibited both anti-collagenase and anti-elastase qualities and considerably inhibited the formation of advanced glycation end products (AGEs) when extracted with 50% ethanol. Additionally, Sang-Yod-SY, an extract from Thai red colored rice, has shown promise as an inhibitor of 5 α -reductase and a natural stimulator of hair growth [12]. Additionally, Hom-Kularb-Dang rice bran extract has shown a protective effect on primary skin fibroblasts exposed to UVB irradiation, increasing type I procollagen synthesis and decreasing matrix metalloproteinase-1 (MMP-1) expression [13].

The middle plains, particularly Suphanburi and Chai Nat, are where RD43 rice, a specialty Thai variety, is primarily produced. Because of its low GI profile, it reduces blood glucose swings through a steady metabolic rate. Beyond its established application as a functional food ingredient [14], RD43 rice has demonstrated significant radical-scavenging activity [15]. These antioxidant properties, particularly in the brown (unpolished) form, suggest a broader potential for bioactive extraction in dermo-cosmetic research. To date, no cosmetic products containing RD43 rice extract have been documented on the market. Consequently, the objective of this study was to explore the possible use of RD43 brown and white rice extracts as natural ingredients in skincare products. The study assessed free radical scavenging activity, cytotoxic effects on normal human dermal fibroblasts (NHDF), and the anti-inflammatory properties of RD43 brown and white rice extracts, with particular emphasis on inhibition of nitric oxide (NO) production. Additionally, the stability of a topical emulsion containing RD43 rice extract was evaluated. This research represents the inaugural comprehensive examination of this subject.

2. Materials and Methods

2.1 Chemicals and Reagents

Gallic acid, Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox, quercetin, xanthine, xanthine oxidase (from bovine milk, E.C.1.17.3.2.), and TPTZ (2,4,6-tri (2-pyridyl)-s-triazine) were purchased from Sigma-Aldrich Co., Ltd, USA. Nitro blue tetrazolium (NBT), thobarbituric acid (TBA), and 2-Deoxy-D-ribose were purchased from Tokyo Chemical Industry Co., Ltd., Japan. Ethanol (AR grade) was purchased from Merck Co., Ltd, Germany. Hydrochloric acid, ferric chloride, and anhydrous sodium sulfate were purchased from Qrec Co., Ltd, New Zealand. Penicillin-streptomycin, fetal bovine serum (FBS), and Dulbecco's Modified Eagle Medium (DMEM) were purchased from Gibco Co., Ltd, USA. Normal human dermal fibroblast (NHDF) cells were acquired from ATCC Co., Ltd, USA. RAW 264.7 macrophage cell line was purchased from Elabscience Co., Ltd, USA. Nitric oxide assay kits were purchased from Thermo Fisher Co., Ltd, Austria. Sterile water was acquired from A.N.B. Laboratories Co., Ltd, Thailand. Griess reagent kits were acquired from Invitrogen Co., Ltd, Austria. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was procured from Amresco Co., Ltd, USA. All chemical compounds utilized in cosmetic formulations were of cosmetic grade.

2.2 Extraction of RD43 rice

RD43 brown and white rice were sourced from Rai Trupchaiarun in Sam Chuk District, Suphanburi Province, Thailand. The RD43 brown and white rice grains were placed in a hot-air oven at 50°C for 24 hours. Following this process, they were ground into a fine powder using a commercial grinder. The powder was then macerated with 70% (v/v) ethanol at a 1:10 (w/v) ratio of dried RD43 rice to solvent [16]. The mixture was allowed to remain at room temperature (30±5°C) for 24 hours. Next, the solvent was filtered through Whatman No. 1 filter paper. The marc was subjected to a second extraction using the same procedure, performed in triplicate. The combined filtrate was concentrated using a vacuum rotary evaporator (Buchi, R205/v, Switzerland) at 45°C. Each crude extract was weighed and subsequently stored in a light-protected, airtight container at 0°C within a refrigerator. The yield of each extract was determined by employing the following equation:

$$\text{Percentage of Yield} = (W_a / W_b) \times 100 \quad (1)$$

where W_a represents the mass of RD43 brown or white rice extract, and W_b denotes the mass of the dehydrated RD43 brown or white rice powder.

2.3 Analysis of bioactive compounds

2.3.1 Total content of phenolic

The total phenolic content in extracts of RD43 brown and white rice was assessed using a modified Folin-Ciocalteu method, as described in prior research [17]. In summary, 25 µL of each extract solution (1.0 mg/mL) was mixed with 125 µL of Folin-Ciocalteu reagent (10% v/v) and permitted to stand at room temperature for 4 minutes before the incorporation of 100 µL of sodium carbonate (Na_2CO_3) (5.0% w/v). After a 30-minute incubation period at room temperature in the absence of light, the absorbance of the solution was measured at 765 nm utilizing a microplate reader (Biochrom, EZ Read 2000, England). The overall phenolic content was measured in milligrams of gallic acid equivalents per gram of extract (mg GAE/g of extract) using a gallic acid standard curve ranging from 0.01 to 0.25 mg/mL. This experiment was performed three times.

2.3.2 Total content of flavonoids

The overall flavonoid concentration in RD43 extracts of both brown and white rice was evaluated by the development of a flavonoid-aluminum complex, as previously examined [18] with minor adjustments. In summary, 25 µL of each extract solution (1.0 mg/mL) was mixed with ethanol (75 µL) and aluminum chloride (AlCl_3 , 10% w/v) (10 µL). Subsequently, potassium acetate (CH_3COOK , 1.0 M) (10 µL) and deionized water (140 µL) were added to the mixture. The resulting solution was mixed well and incubated for 40 minutes in the dark at ambient temperature. Absorbance was measured at 415 nm using a microplate reader (Biochrom, EZ Read 2000, England). The overall flavonoid concentration was measured in milligrams of quercetin

equivalent per gram of extract (mg QE/g of extract), utilizing the calibration curve (0.0012-0.3125 mg/mL). This analysis was conducted in triplicate.

2.3.3 Content of gamma-oryzanol

Gamma-oryzanol levels in RD43 brown rice and white rice extracts were measured using derivative UV spectrophotometry, as outlined in prior studies [19], with specific alterations. The absorbance of each extract solution (1.0 mg/mL) was measured at 315 nm utilizing a UV-visible spectrophotometer (Shimadzu, UV-2401PC, Japan). The level of gamma-oryzanol in the extracts from RD43 brown and white rice was quantified in milligrams of gamma-oryzanol per gram of extract (mg/g of extract), based on the calibration curve (0.00625-0.02 mg/mL). This experiment was conducted three times.

2.3.4 The phenolic acids and flavonoids profile

The phenolic acid and flavonoid profiles in extracts derived from RD43 brown and white rice were analyzed using high-performance liquid chromatography (HPLC), following the protocol established in previous studies [20] and [21], with minor modifications. Phenolic acids and flavonoids were identified using an Agilent 1200 HPLC system (Agilent Technologies, USA) equipped with a P-4000 pump (Thermo Separation Products-TSP, Riviera Beach, USA). The chromatographic separation was performed using a C18 column (4.6 mm x 150 mm, 5 μ m) from Phenomenex, Torrance, CA, USA. The column was kept at room temperature while maintaining a steady flow rate of 1.0 mL/min, and an autosampler injected 10 μ L of each sample individually into the column.

For phenolic acid analysis, the mobile phases were (A) acetic acid (1.0% v/v) and (B) acetonitrile. The gradient elution was executed as follows: from 0 to 5 minutes, a linear gradient was applied, increasing from 5% to 9% solvent B; from 5 to 15 minutes, solvent B was held constant at 9%; from 15 to 22 minutes, a linear gradient was again applied, this time from 9% to 11% solvent B; from 22 to 38 minutes, the gradient continued linearly from 11% to 18% solvent B; from 38 to 43 minutes, the gradient shifted from 18% to 23% solvent B; and finally, from 43 to 44 minutes, the gradient increased from 23% to 90% solvent B. A 6-minute wash with 80% solvent B was followed by a 15-minute re-equilibration with 5% solvent B between each run. An analysis of flavonoid compounds was performed utilizing gradient elution with two solvents: (A) a formic acid solution at pH 2.5 and (B) methanol. The gradient elution was executed as follows: from 0 to 20 minutes, a linear gradient transitioned from 80% to 30% solvent A; from 20.1 to 22 minutes, an isocratic condition at 90% solvent B was maintained, followed by an 8-minute re-equilibration period using 80% solvent A between individual runs. The column was kept at ambient temperature with a consistent flow rate of 1.0 mL/min, and an autosampler injected 10 μ L of each sample separately. Each standard phenolic acid and flavonoid compound was injected separately to assess retention times (RT). The phenolic acids (including gallic acid, protocatechuic acid, vanillic acid, caffeic acid, coumaric acid, ferulic acid, and sinapic acid) and flavonoids (such as catechin, rutin, and quercetin) were identified at a wavelength of 280 nm. This study was carried out three times.

2.4 Evaluation of cytotoxicity to normal human dermal fibroblasts

Cytotoxicity of RD43 brown and white rice extracts was evaluated to assess the viability of cultured fibroblast cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, following the methodology described in earlier research [22] with specific modifications. In summary, normal human dermal fibroblast (NHDF, ATCC, PCS-201-010) cells were utilized as the cellular model to examine cytotoxicity. The fibroblast cells were trypsinized and plated at approximately 8×10^3 cells per well in a 96-well plate. They were then cultured in complete growth medium, consisting of DMEM-F12 enriched with fetal bovine serum (FBS) at 10% v/v and penicillin/streptomycin at 1.0% v/v. The fibroblast cells were maintained at 37°C in a humidified environment with 5% CO₂ for 24 hours. After this incubation phase, the culture medium was discarded, and the cells were rinsed with sterile phosphate-buffered saline. The fibroblast cells were then exposed to different concentrations of crude extracts (5 to 100 mg/mL), compared with the control group (untreated cells). After an additional 24 hours of incubation, the media containing the samples were removed, and the cells were rinsed once more with sterile phosphate-buffered saline. Next, the standard human dermal fibroblast cells were treated with MTT solution and maintained at 37°C in the dark for 2 hours.

Subsequently, the medium was discarded, and the formed formazan crystals were dissolved in dimethyl sulfoxide. The absorbance for each well was assessed with a microplate reader set to 570 nm. The calculation of cell viability percentage was performed using the equation presented below:

$$\text{Percentage of cell viability} = (A_a/A_b) \times 100 \quad (2)$$

where A_a represents the absorbance of the sample under investigation, and A_b denotes the absorbance of the control, which is the cell that has not been treated with the sample in question.

2.5 Assessment of antioxidant activities in vitro

2.5.1 DPPH radical scavenging assay

The DPPH radical scavenging activity of extracts from RD43 brown and white rice was evaluated following the methodology described in earlier research [13], with minor adjustments. In brief, 75 μL from various concentrations of each extract solution were mixed with 150 μL of DPPH ethanolic solution (0.2 mM). The final mixture was allowed to stand at room temperature in the dark for thirty minutes. After the incubation period, absorbance at 515 nm was measured using a microplate reader (Biochrom, EZ Read 2000, England). This study was compared using Trolox, the positive control. The proportion of DPPH radical scavenging activity was computed using the provided formula:

$$\text{Percentage of DPPH radical scavenging activity} = [(A_b - A_s)/A_b] \times 100 \quad (3)$$

where A_b indicates the absorbance of DPPH in the absence of the tested sample and A_s indicates the absorbance of DPPH in the presence of the tested sample. The graph showing the proportion of inhibition against the sample concentration was used to calculate the concentration that causes 50% inhibition (IC_{50}). Three runs of the experiment were conducted.

2.5.2 Ferric reducing antioxidant power (FRAP) evaluation

With a few minor adjustments, the ferric reducing antioxidant power (FRAP) assay was carried out using the approach outlined in previous studies [18]. Acetate buffer (300 mM, pH 3.6), 10 mM TPTZ, and 20 mM ferric chloride (FeCl_3) were first combined in a 10:1:1 ratio to create the FRAP solution. The FRAP solution (190 μL) was then combined with 10 μL of each extract concentration (1.0 mg/mL). Absorbance was recorded at 593 nm (EZ Read 2000, Biochrom) immediately after the samples were equilibrated at 37°C for 30 minutes. A linear regression equation derived from a Trolox standard 0.03125 to 1.0 mg/mL was employed for data interpolation. The final antioxidant activity is reported as mg TE/g of dry extract. This experiment was carried out three times

2.5.3 Evaluation of Hydroxyl radical scavenging activity (HRSA)

A modified deoxyribose assay was used to assess the RD43 brown and white rice extracts' capacity to scavenge hydroxyl (OH) radicals [23]. Ferric chloride (0.3 mM), L-ascorbic acid (0.6 mM), EDTA (1.2 mM), H_2O_2 (34 mM), and deoxyribose (17 mM) were used in a 2:2:1:1:1 ratio in a Fenton-type reaction to produce hydroxyl radicals. Add 30 μL of each extract (1.0 mg/mL) to a 210 μL aliquot of this mixture. After 30 minutes at 37°C, 2.8% (v/v) trichloroacetic acid (TCA, 300 μL) and 1.0% (w/v) thiobarbituric acid (TBA, 150 μL) were added to stop the reaction. The TBA-malonaldehyde chromophore was developed by heating the combination to 100°C for five minutes. The absorbance was measured at 532 nm (EZ Read 2000, Biochrom, England). Results were interpolated from a Trolox standard curve (0.15625–5.0 mg/mL) and expressed as mg TE/g extract. All measurements were performed in triplicate.

2.5.4 Evaluation of Superoxide radical scavenging activity (SRSA)

The ability of RD43 brown and white rice extracts to scavenge superoxide radicals was assessed following the methodology outlined in previous studies [23], with minor adjustments. Initially, the superoxide radical was produced by mixing 75 μL of 0.3 mM xanthine, 50 μL of 0.15 mM nitroblue tetrazolium (NBT), 50 μL of 0.6 mM EDTA, and 25 μL of xanthine oxidase (1.0% w/v, 5 units/mL). Subsequently, 7.5 μL of each extract concentration (1.0 mg/mL) was added to the mixture in a 96-well plate. After incubation for 40 minutes at 37°C, absorbance was measured at 560 nm using a microplate reader (Biochrom, EZ Read 2000, England).

The findings were expressed as milligrams of trolox equivalent per gram of extract (mgTE/g of extract), based on the standard curve of trolox (0.15625-5.0 mg/mL). The experiment was performed three times.

2.6 In vitro assessment of anti-inflammatory potential

2.6.1 Murine macrophage RAW 264.7 cell culture

RAW 264.7 cells (1×10^4 cells/well) were cultured in 96-well plates using RPMI-1640 medium (10% FBS, 1% penicillin/streptomycin). After seeding, the plates were incubated for 24 hours at 37°C in a 5% CO₂ environment before experimental intervention.

2.6.2 Cytotoxicity

The MTT assay was used to assess cell viability [24]. For 24 hours, RAW 264.7 macrophages were cultured at 1×10^4 cells/well in a humidified incubator (37°C, 5% CO₂). To check for cytotoxic effects, the cells were exposed to different doses of the rice extracts after this pre-incubation phase. Each extract of RD43 brown and white rice was given at diverse doses, ranging from 0 to 250 µg/mL, after this incubation. As the vehicle control, dimethyl sulfoxide ensured that the final concentration remained below 0.2% v/v. After a further 24-hour incubation, the spent media was disposed of. Next, 20 µL of MTT reagent (5.0 mg/mL) was added to each well, and the mixture was incubated for 2 hours at 37°C in a CO₂ incubator. Each well's absorbance at 570 nm was measured using a microplate reader (Synergy H1, BioTek, USA).

2.6.3 Inhibition of nitric oxide (NO) production

A 96-well plate was used to plate the murine macrophage cell line RAW 264.7 at a density of 5×10^4 cells per well. [25] The cells were then incubated for a full day. RAW 264.7 cells were pretreated with RD43 extracts (0-25 µg/mL) for 30 minutes before LPS stimulation (1.0 mg/mL). After a 24-hour incubation at 37°C and 5% CO₂, supernatants were collected to quantify inflammatory markers. Using the Griess reagent kit and the Griess assay [26], nitric oxide release in the culture supernatant was assessed after 30 minutes of incubation at room temperature. A microplate reader (Synergy H1, BioTek, USA) was used to measure nitric oxide at 570 nm. Compared with the nitrite levels obtained from the dimethyl sulfoxide control in cells treated with LPS, results were expressed as nitrite concentrations (µM). 50 µM of indomethacin served as a positive control.

2.7 Preparation of the oil-in-water (O/W) emulsion

A hot-process emulsification technique was employed to engineer the O/W topical emulsion. Heating both phases to 70–75°C before homogenization produced stable oil-in-water (O/W) emulsions. This procedure optimized droplet-size distribution and ensured the internal phase's long-term structural integrity. The lipid and aqueous phases (deionized water, disodium EDTA, propylene glycol, glycerin, butylene glycol, and sodium polyacrylate) were prepared independently, then heated to 75°C to create the emulsion vehicle. The formulation was homogenized at 1,500 rpm for 15 minutes after the lipid phase was integrated into the aqueous medium (Silverson L5M, England). Achieving the required rheological characteristics and long-term structural uniformity required this high-shear procedure. The emulsion was then gradually cooled to room temperature (35±5°C). To complete the emulsion, phenoxyethanol (0.5% w/w) and a mixture (2.0% w/w) of propanediol and ethylhexylglycerin were added as preservative agents. RD43 brown rice (0.2% w/w) was well solubilized with polyethylene glycol 400 before being added to the emulsion base at 40°C. A comparative analysis of the physicochemical and organoleptic properties was performed for both the control cream base and the RD43 brown rice extract formulation. Evaluation parameters included macroscopic appearance, structural homogeneity, sensory skin feel, pH, and viscosity.

2.8 Physicochemical stability studies

Stability assessments of the skin care formulations were conducted under different conditions, including room temperature (30±5°C), cold temperature (4±1°C), and elevated temperature (45±1°C), over 30 days. Accelerated aging was simulated via six H/C cycles (45°C/4°C, 24 h each). Subsequently, the organoleptic and physical characteristics of the formulations, ranging from sensory attributes like skin feel to technical metrics such as pH and phase stability, were rigorously assessed to ensure formula robustness.

2.9 Statistical analysis

Results are presented as the mean $\pm$ SD ($n=3$). Differences between treatment groups were evaluated using one-way ANOVA, and mean separations were determined using Duncan's multiple range test. Statistical processing was executed using IBM SPSS Statistics for Windows (Version 22.0), and a p-value of less than 0.05 was considered statistically significant.

3. Results and Discussion

3.1 Percentage yield of RD43 rice extracts

Solvent extraction is the predominant technique used to extract plant materials, with numerous studies focusing on environmentally friendly solvents. Ethanol and water are recognized as green, sustainable solvents, and when paired with low-environmental-impact technologies, they offer a promising strategy for developing eco-friendly extraction processes. Conversely, the maceration method is a straightforward extraction technique that requires minimal energy and is cost-effective [27]. In this study, maceration with 70% ethanol was used to obtain extracts from the dry powders of both brown and white RD43 rice. The physical characteristics of the crude extracts from RD43 brown and white rice were assessed visually. RD43 brown and white rice extracts both appeared brownish with medium stickiness; however, the extractive yield of the brown rice variant (3.06%) was more than double that of the white rice variant (1.28%). The observed variation in yield is likely attributable to the hydroalcoholic solvent system (70% v/v ethanol), which optimizes the extraction of phytochemicals with varying polarities. This system effectively facilitates the solubilization of compounds from the RD43 brown rice matrix that are soluble in both aqueous and organic phases, including phenolic acids (e.g., ferulic, sinapic, and p-coumaric acids), flavonoids (e.g., luteolin and apigenin), and proanthocyanidins [9]. Additionally, other compounds may have been extracted, contributing to the higher yield.

3.2 Bioactive compound analysis

Building on evidence that rice bioactives are governed by genotype and tissue type [9], this study characterized the total phenolic, flavonoid, and gamma-oryzanol profiles of RD43 rice extracts. By assessing these specific phytochemical constituents, we evaluated the suitability of RD43 brown and white rice as functional ingredients for photoprotective cosmetic products. RD43 brown rice is a whole-grain caryopsis, while white rice is the polished endosperm of a similar cultivar. The whole-grain caryopsis is structurally composed of bran (pericarp and aleurone layers), germ (embryo), and starchy endosperm. By removing the bran and germ through milling and polishing, white rice differs chemically from brown rice because it consists primarily of carbohydrate-rich endosperm. The total phenolic, flavonoid, and gamma-oryzanol contents in RD43 brown and white extracts were summarized in Table 1. Results: The highest levels of total phenolics (20.03 ± 0.42 mgGAE/ g of extract), total flavonoids (13.37 ± 0.72 mgQE/g of extract), and gamma-oryzanol (14.62 ± 0.03 mg/g of extract) were observed in the RD43 brown rice extract, where these values were significantly higher ($p<0.05$).

Table 1. Contents of total phenolic, total flavonoid, and gamma-oryzanol of RD43 rice extracts

Crude extracts	Total phenolic (mg GAE/g of extract)	Total flavonoid (mg QE/g of extract)	gamma-Oryzanol (mg/g of extract)
RD43 brown rice	20.03 ± 0.42^a	13.37 ± 0.72^a	14.62 ± 0.03^a
RD43 white rice	5.26 ± 0.65^b	6.28 ± 0.22^b	5.33 ± 0.01^b

Data are presented as mean $\pm$ standard deviation ($n = 3$). Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$).

Figures 1-3 present the HPLC chromatograms for seven phenolic acid standards, three flavonoid standards, and the RD43 rice extracts (brown and white). Table 2 provides detailed quantitative analysis of these phytochemicals.

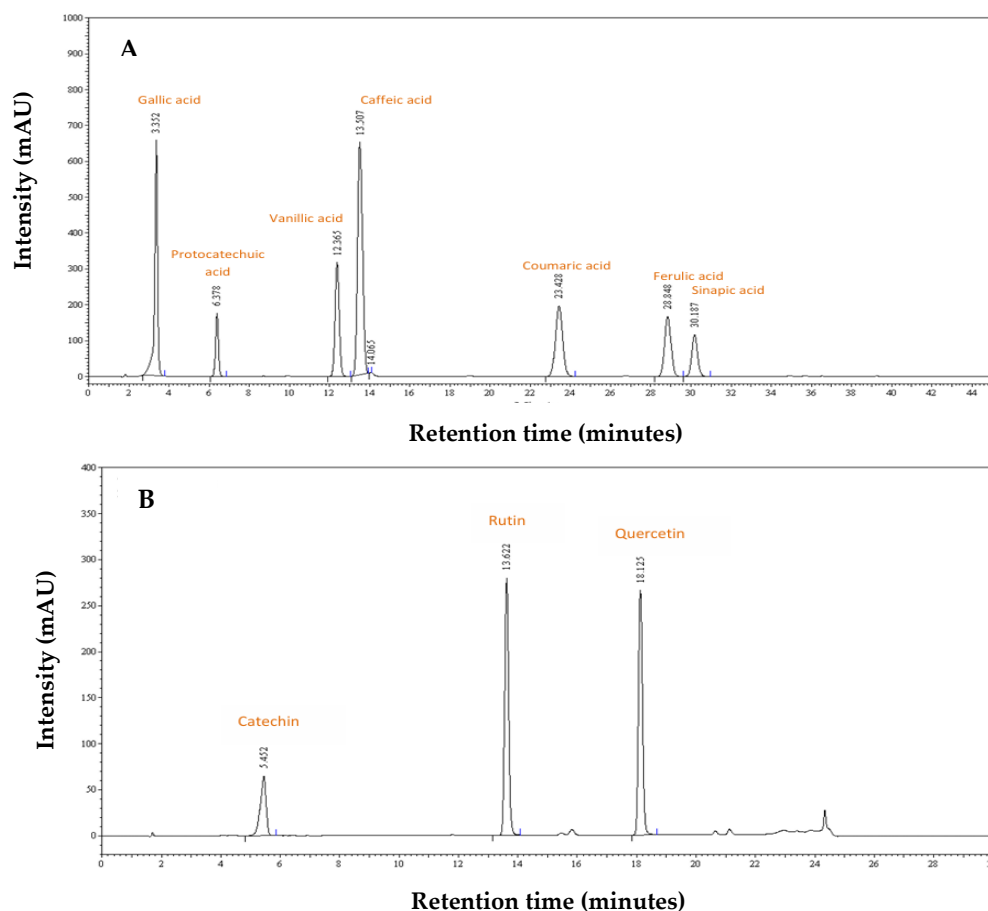


Figure 1. HPLC profiles of authentic standards, showing the separation of (A) seven phenolic acids and (B) three flavonoids

Table 2. Quantitative analysis of phenolic acid and flavonoid profiles in RD43 brown and white rice extracts

Compounds		Retention time (RT, minutes)	Amount ($\mu\text{g/g}$ of extract)	
			RD43 brown rice extract	RD43 white rice extract
Phenolic acids	Gallic acid	3.352	43.72 ± 0.89^g	10.19 ± 0.84^h
	Protocatechuic acid	6.378	521.10 ± 27.50^e	92.98 ± 12.38^{ef}
	Vanillic acid	12.365	516.50 ± 42.20^e	119.10 ± 36.17^{de}
	Caffeic acid	13.507	95.00 ± 4.49^f	41.16 ± 6.35
	Coumaric acid	23.428	605.40 ± 24.30^d	103.50 ± 9.28^{de}
	Ferulic acid	28.848	808.20 ± 26.20^c	44.59 ± 3.39^g
	Sinapic acid	30.187	813.10 ± 3.12^c	1070.50 ± 82.10^b
Flavonoids	Catechin	5.452	7610.80 ± 249.30^a	3660.80 ± 115.70^a
	Rutin	13.622	1533.70 ± 74.10^b	146.10 ± 10.74^d
	Quercetin	18.125	15.06 ± 3.13^h	335.50 ± 3.13^c

All experiments were performed in triplicate ($n = 3$), and results are expressed as mean $\pm$ SD. Different superscript letters within the same column indicate statistically significant differences among the identified phenolic acids and flavonoids within each extract ($p < 0.05$).

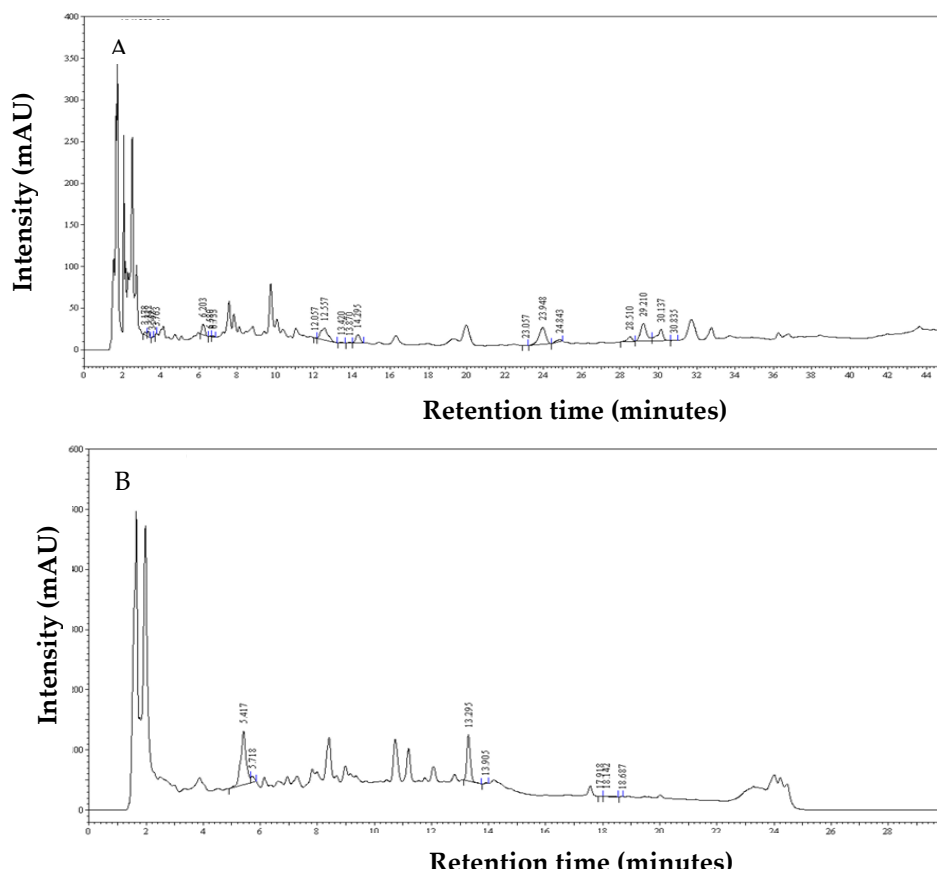


Figure 2. HPLC chromatogram of RD43 brown rice extract; (A) phenolic acids and (B) flavonoids

These findings are consistent with previous reports indicating that ferulic, p-coumaric, and cinnamic acids are the predominant phenolic acids across the bran, endosperm, and whole-grain fractions of non-pigmented rice. Additionally, the flavonoid profile is characterized primarily by flavones, most notably apigenin and tricetin. [9]. In this study, the HPLC chromatogram showed that the RD43 brown rice extract exhibited the highest concentrations of ferulic acid among phenolic acids and catechin among flavonoids ($p < 0.05$). In contrast, sinapic acid and catechin were found in the highest concentrations ($p < 0.05$) among the phenolic acids and flavonoids, respectively, in the RD43 white rice extract. Interestingly, the white RD43 rice extract exhibited a higher sinapic acid content than the corresponding brown rice extract. Although brown rice is generally recognized as a richer source of total phenolic compounds due to the presence of bran and germ layers [9,20], the distribution of individual phenolic acids may vary depending on cultivar, grain fraction, and chemical form [39]. Phenolic acids occur as free, conjugated, and bound forms, with a substantial proportion existing in bound forms associated with cell wall polysaccharides [40, 41]. Therefore, differences in extraction efficiency may influence the recovery of specific compounds. The higher sinapic acid content observed in white RD43 rice may be attributed to a greater proportion of extractable free sinapic acid or reduced extraction of bound sinapic acid from the bran-rich matrix of brown rice. Similar observations have been reported in cereal grains, where the measured concentrations of individual phenolic acids were strongly influenced by their extractability and chemical association with grain components [40, 41]. This finding suggests that the concentration of individual phenolic acids does not necessarily follow the same pattern as total phenolic content and may represent a cultivar-specific characteristic of RD43 rice [9, 39]. These results are consistent with prior studies [9, 28–30], which demonstrated that brown rice extracts possess significantly higher total phenolic and flavonoid contents compared to white rice extracts. Characterized as a mixture of steryl ferulates with robust antioxidant activity [31], gamma-oryzanol is found throughout the rice grain, including the bran and endosperm. This substance provides significant therapeutic benefits, including

improved lipid profiles and management of glycemic levels in diabetic patients through increased insulin production [32]. Consistent with prior research, germination was found to increase gamma-oryzanol content, with germinated brown rice extracts surpassing those of non-germinated brown rice [30]. The results confirmed that the total gamma-oryzanol content in RD43 brown rice extract was higher than in white rice extract.

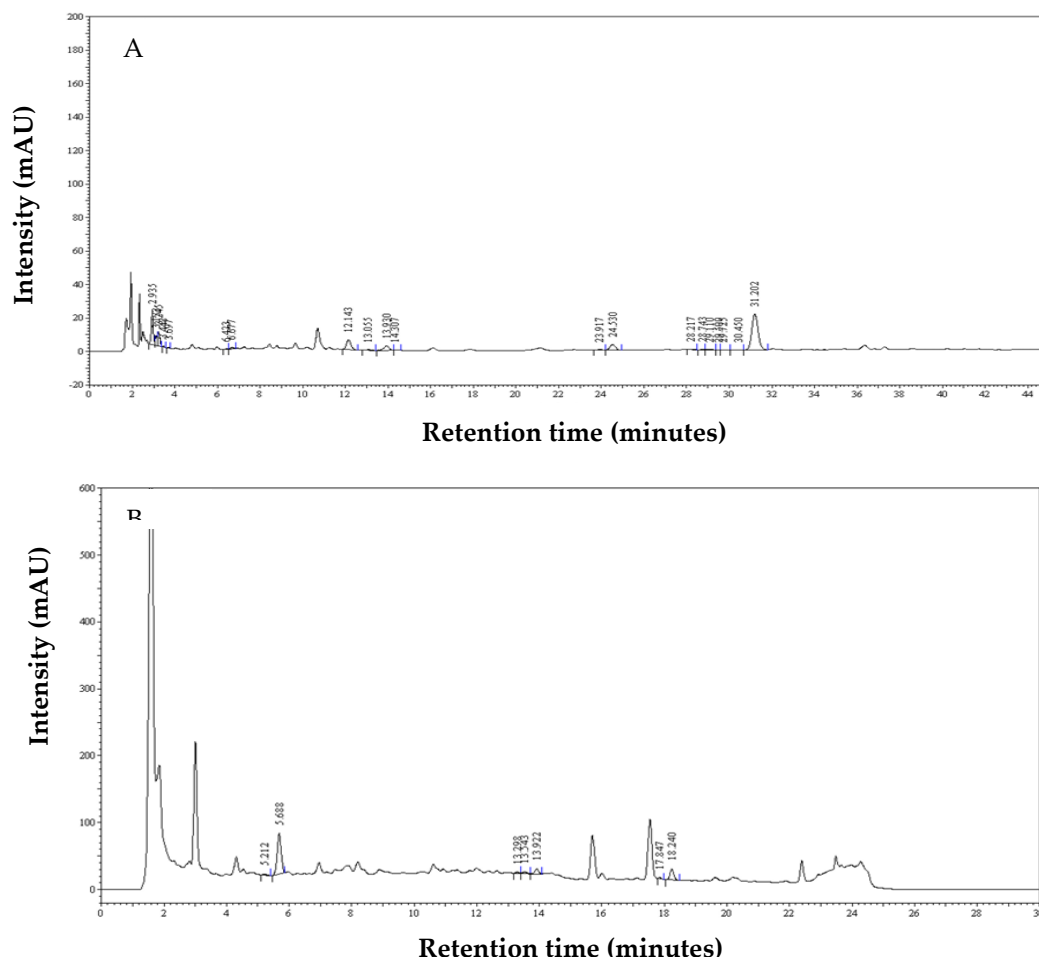


Figure 3. HPLC chromatogram of RD43 white rice extract; (A) phenolic acids and (B) flavonoids

3.3 Cytotoxicity effect on normal human dermal fibroblasts (NHDF) cells

One criterion for assessing the cytotoxicity of the crude extract is cell viability. Cytotoxicity assessments via MTT assay revealed that RD43 rice extracts (5.0–100 µg/mL) did not adversely affect NHDF cell viability. With cell viability exceeding 90% across all treatments after 24 hours (Figure 4), both extracts are non-toxic to NHDF cells within the evaluated concentration range in vitro. and compared to the control group ($p < 0.05$)

3.4 In vitro antioxidant activities

Oxidative damage is a consequence of free radicals. Antioxidants act as reducing compounds, reducing damage to biological structures by donating electrons to free radicals and eliminating their reactivity [33]. As plant-derived phenolics and flavonoids act as effective antioxidants, this study investigates the scavenging activity of RD43 brown and white rice extracts for application in topical cosmetic products. The antioxidant capacities of RD43 brown and white rice extracts were determined using DPPH, FRAP, HRS, and SRS (Table 3). The DPPH assay measured antioxidant efficiency. RD43 extracts caused a colorimetric change from purple to yellow, indicating the creation of stable non-radicals. Both extracts demonstrated dose-dependent scavenging; RD43 brown rice ($IC_{50} = 1.90 \pm 0.05$ mg/mL; $p < 0.05$) performed noticeably better than RD43 white rice ($IC_{50} = 3.98 \pm 0.27$ mg/mL). The extracts' performance aligns with the traits of crude botanical

samples, though they are less effective than the Trolox standard. To further enhance antioxidant efficacy, future research should aim to isolate and characterize the bioactive constituents responsible for the observed activity. Evaluation of purified fractions and individual compounds may reveal higher antioxidant potency than that observed for the crude extract. The FRAP assay, which measures the reduction of the Fe(III)-tripyridyltriazine (TPTZ) complex to its blue Fe(II) form, was employed to determine the total antioxidant capacity of the extracts. Results indicated that the RD43 brown rice extract exhibited a significantly higher ferric reducing power (374.12 ± 2.75 mg TE/g of extract) than the RD43 white rice extract ($p < 0.05$), indicating a high concentration of reductone-like bioactive compounds.

Hydroxyl (OH) radicals are the most reactive and predominant radicals generated endogenously during aerobic metabolism to initiate cell damage [34]. The antioxidant capacity of RD43 rice extracts was evaluated by monitoring their ability to inhibit the oxidative degradation of deoxyribose, thereby quantifying their efficiency in neutralizing hydroxyl radicals. Our results also showed that RD43 brown rice extract exhibited better hydroxyl radical scavenging than RD43 white rice extract, with an activity value of 223.77 ± 3.27 mg TE/g of extract. Superoxide anion is an essential upstream radical in cellular redox biology. Despite its modest reactivity, it acts as a substrate for the production of additional harmful species, such as singlet oxygen and hydroxyl radicals. As a result, scavenging superoxide is an essential cytoprotective mechanism that stops the harmful consequences of long-term oxidative stress [35]. The results of the experiment demonstrated that RD43 brown rice extract, with an active value of 176.42 ± 6.01 mg TE/g of extract, could prevent superoxide formation more than RD43 white rice extract.

The antioxidant activity observed in RD43 rice extracts is likely attributable to the combined effects of phenolic acids, flavonoids, and γ -oryzanol. Phenolic acids, particularly ferulic acid and sinapic acid, are well known for their ability to donate hydrogen atoms or electrons to neutralize free radicals and terminate oxidative chain reactions. Their antioxidant activity is associated with hydroxyl and methoxy groups that stabilize phenoxy radicals through resonance structures [9-11]. Flavonoids such as catechin also contribute significantly to antioxidant activity through radical scavenging, metal-chelating, and reducing mechanisms. In addition to their antioxidant properties, catechins have been reported to modulate inflammatory responses by suppressing the production of pro-inflammatory mediators, including nitric oxide, and by regulating signaling pathways such as NF- κ B and MAPK [10, 16]. Furthermore, γ -oryzanol, a characteristic bioactive compound in rice, has been reported to exhibit antioxidant and anti-inflammatory activities by inhibiting lipid peroxidation and reducing oxidative stress-induced cellular damage. γ -Oryzanol may also help suppress inflammatory mediator production by modulating intracellular redox balance and inflammatory signaling pathways [31]. Therefore, the antioxidant and anti-inflammatory activities observed in RD43 brown rice extract likely result from synergistic interactions among multiple bioactive constituents rather than the action of a single compound. These findings highlight its usefulness as a functional ingredient in topical emulsion products that offer protection.

Table 3. In vitro antioxidant activities of RD43 brown and white rice extracts

Extract/ Positive control	DPPH (IC ₅₀ mg/mL)	FRAP assay (mgTE/g)	HRS assay (mgTE/g)	SRS assay (mgTE/g)
RD43 brown rice	1.90 ± 0.05^b	374.12 ± 2.75^a	223.77 ± 3.27^a	176.42 ± 6.01^a
RD43 white rice	3.98 ± 0.27^a	60.38 ± 2.18^b	130.38 ± 2.47^b	46.78 ± 1.16^b
Trolox	0.08 ± 0.001^c	-	-	-

Results are presented as mean $\pm$ SD (n = 3). Statistically significant differences ($p < 0.05$) between experimental groups are indicated by non-identical superscript letters.

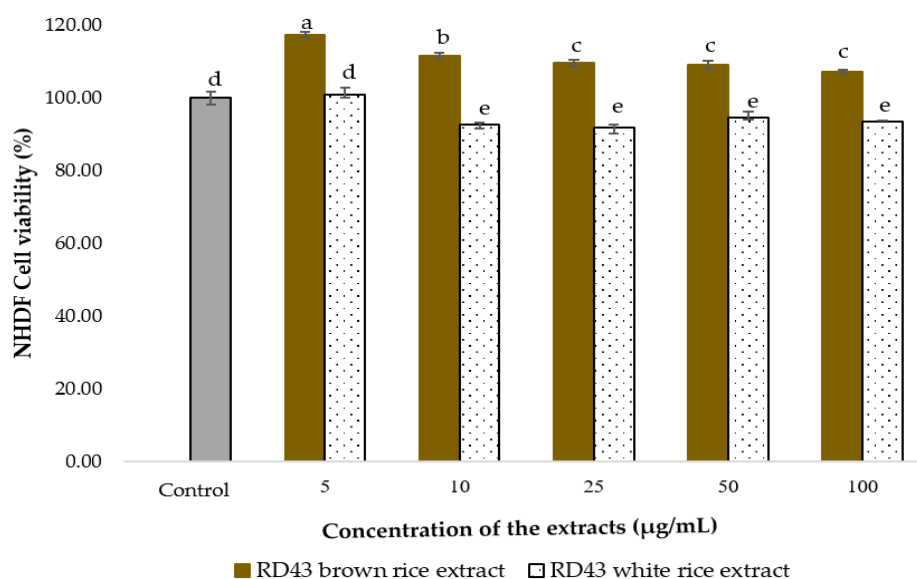


Figure 4. Percentage of normal human dermal fibroblast (NHDF) cell viability after exposure to RD43 brown and white rice extracts for 24 hours. Bars indicate the mean $\pm$ SD of triplicate measurements. Distinct letters above the bars signify statistical significance between concentrations within the same extract

3.5 In vitro assessment of anti-inflammatory potential

Using the MTT test, cytotoxicity was evaluated in RAW264.7 cells treated with RD43 brown rice and white rice extracts at different concentrations (5.0–250 µg/mL), as shown in Figure 5. Compared with the untreated group (100% cell viability), cell viability for both extracts was assessed. The results show that both extracts reduced cell viability in a concentration-dependent way. The RD43 white rice extract exhibited significant cytotoxicity ($p < 0.05$) at a broader concentration range (25–250 µg/mL) compared to the brown rice extract, which showed significant inhibitory effects starting at 50 µg/mL. However, both extracts maintained cell viability above 90% at lower dosage levels, indicating high cellular biocompatibility within those ranges.

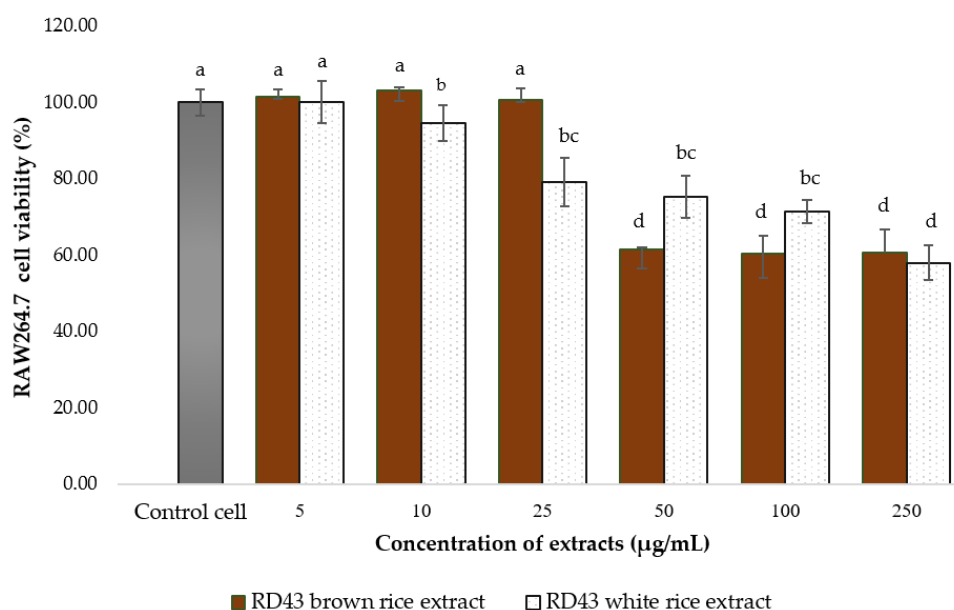


Figure 5. Percentage of RAW264.7 cell viability after exposure to RD43 brown and white rice extracts for 24 hours. Each bar represents the mean $\pm$ SD (n = 3). Within each extract group, bars labeled with different letters differ significantly from the control ($p < 0.05$).

While macrophages are essential mediators of immune function, dysregulated nitric oxide (NO) secretion contributes significantly to the onset of chronic inflammatory diseases. High concentrations of NO induce oxidative damage, potentially resulting in mutagenesis and neuronal impairment through the formation of reactive nitrogen species [36]. Consequently, suppressing NO biosynthesis may attenuate the inflammatory response, thereby reducing the risk of chronic disorders associated with oxidative and nitrosative stress. Figure 6 presents the quantification of nitrite (NO_2^-) accumulation in LPS-stimulated RAW 264.7 macrophages following treatment. NO production was determined by the Griess reaction, which measures the concentration of nitrites released into the extracellular medium. The ability of RD43 rice extracts to inhibit pro-inflammatory mediators was investigated by measuring nitrite accumulation in LPS-activated RAW 264.7 cells. The LPS-induced inflammatory response resulted in a baseline NO concentration of $16.52 \pm 0.41 \mu\text{M}$. Treatment with indomethacin ($1.27 \pm 0.53 \mu\text{M}$) successfully suppressed this production ($p < 0.05$), providing a potent reference point for comparing the dose-dependent inhibitory activities of the RD43 rice extracts. Both extracts demonstrated a dose-dependent suppression of nitric oxide (NO) production. While the RD43 brown rice extract at $25 \mu\text{g/mL}$ did not show a significantly greater inhibition ($p < 0.05$) compared to other tested concentrations, it did achieve a statistically significant reduction ($p < 0.05$) in NO levels relative to the RD43 white rice extract. Although effective, the inhibitory potency of both extracts remained lower than that of indomethacin, the positive control.

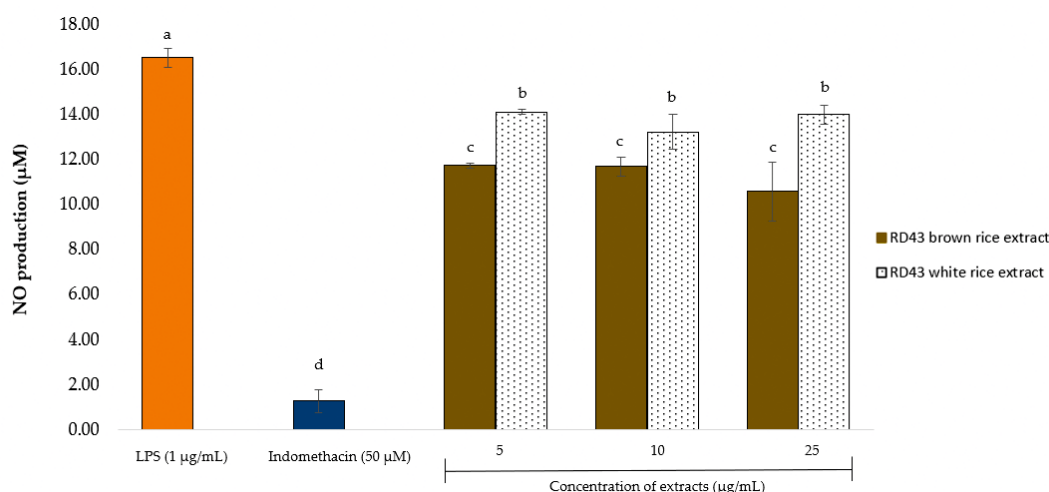


Figure 6. Inhibition of nitric oxide (NO) production in RAW264.7 macrophage cells following 24 hours of treatment with lipopolysaccharide (LPS), indomethacin, or RD43 extracts. Data are expressed as the mean $\pm$ standard deviation (SD) of triplicate measurements ($n=3$). Within each sample group, non-identical superscript letters denote statistically significant differences ($p < 0.05$).

The NO inhibition assay was performed using extract concentrations (0–25 $\mu\text{g/mL}$) that were confirmed to be non-cytotoxic to RAW264.7 cells. Assessing nitric oxide production at non-cytotoxic concentrations is essential to ensure that the observed reduction in NO levels reflects modulation of inflammatory responses rather than a decrease in viable cell number caused by cytotoxic effects [37]. The significant reduction in NO levels suggests that RD43 brown rice extract modulates the inflammatory response. This bioactivity is consistent with the presence of phenolic and flavonoid compounds, which have been extensively characterized for their ability to downregulate pro-inflammatory signaling pathways and neutralize reactive nitrogen species [42]. Therefore, the inhibition of NO production observed in this study can be attributed to the anti-inflammatory potential of the rice extracts rather than impairment of macrophage viability. Although the antioxidant properties of rice-derived bioactive compounds have been extensively reported, most studies have focused on pigmented rice varieties, such as black and red rice, which are rich in anthocyanins and phenolic compounds. Compared with these varieties, RD43 brown rice is non-pigmented; however, the present study showed that its extract contained appreciable levels of phenolic compounds and

flavonoids, particularly ferulic acid and catechin, and exhibited significant antioxidant and anti-inflammatory activities. Similar to reports on rice bran and pigmented rice extracts, the bioactivity of RD43 rice appears to be associated with its phenolic constituents, which contribute to free radical scavenging and anti-inflammatory effects [9-12]. These findings suggest that RD43 rice may serve as an alternative source of natural bioactive compounds for cosmetic applications, despite lacking the anthocyanin-rich profile typically associated with highly antioxidant rice varieties.

3.6 Oil in water (O/W) emulsion

The RD43 brown rice extract was selected for cream formulation due to its higher phenolic and flavonoid contents and superior antioxidant activity compared with the other extracts. An extract concentration of 0.2% (w/w) was chosen to provide sufficient bioactive compounds while maintaining acceptable physicochemical properties, formulation homogeneity, and stability. PEG-400 was incorporated as a co-solvent and solubilizing agent to enhance the dispersion and solubility of rice-derived phytochemicals within the emulsion system. In addition, PEG-400 improved formulation uniformity and contributed to the cream's physical stability by preventing precipitation of poorly soluble constituents during storage [43]. The formulation of the topical emulsion base (without extract) and the cream incorporating RD43 brown rice extract exhibited favorable physical properties, including homogeneity, absence of separation, a white hue (for the cream without extract), and a light-yellow hue (for the cream with extract), as determined through visual assessment (see Figure 7). Both formulations provided a pleasant sensation upon application to the skin. The pH of topical skincare formulations is an important quality parameter because products with pH values close to that of the skin are generally better tolerated and less likely to disrupt the skin barrier. Human skin typically exhibits a slightly acidic surface pH ranging from approximately 5.0 to 5.8 [38]. In the present study, the pH values of the cream base and the cream containing RD43 brown rice extract were 7.03 ± 0.06 and 6.77 ± 0.34 , respectively, with no significant difference between the formulations ($p > 0.05$). Although slightly higher than the natural skin surface pH, both formulations remained within an acceptable range for topical cosmetic products and are unlikely to cause skin irritation or adversely affect product performance. These results indicate that incorporating the extract did not significantly alter the formulation pH and suggest acceptable compatibility for topical application.

Viscosity is another critical quality attribute affecting product spreadability, texture, consumer acceptance, and physical stability. Viscosity measurements for the cream base (without extract) and the cream with extract were 5540.67 ± 145.01 cP and 5096.67 ± 160.35 cP, respectively, indicating a significant difference ($p < 0.05$). This variation in viscosity can be attributed to the inclusion of sodium polyacrylate as a stabilizing agent in the external aqueous phase.

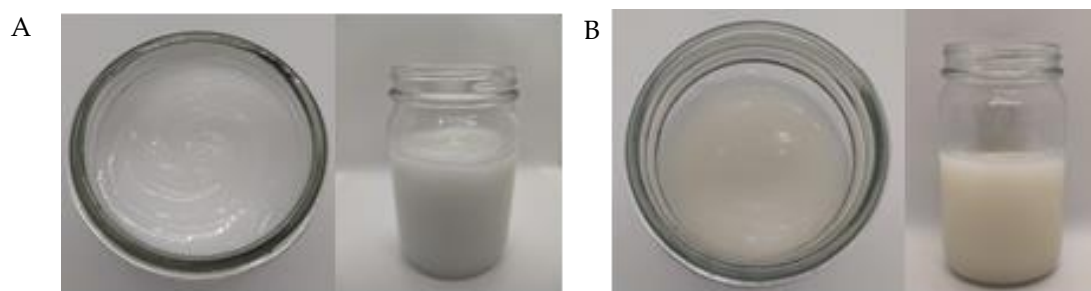


Figure 7. Physical appearance of the (A) cream base formulation (control, without extract) and (B) cream formulation incorporated with RD43 brown rice extract

The physicochemical stability of the cream formula base (without extract) and the cream containing RD43 brown rice extract was assessed by conducting tests at room temperature (RT, $30 \pm 5^\circ\text{C}$), low temperature (LT, $4 \pm 1^\circ\text{C}$), and high temperature (HT, $45 \pm 1^\circ\text{C}$) over a period of 30 days. To assess accelerated physical stability, the products underwent six successive H/C cycles. This protocol involved maintaining the samples at 45°C (24 h) and then at 4°C (24 h) per cycle, allowing observation of potential phase separation or textural

changes under thermal stress. The changes in the products before and after testing were analyzed based on texture, color, odor, skin feel, viscosity, and acid-base (pH) characteristics, with the results presented in Table 4.

Table 4. Stability testing results of topical emulsion containing RD43 brown rice extract

Conditions	Parameters					
	Homogeneity	Phase separation	Color	Feel on skin	pH value	Viscosity (cP)
Initial	Good	No	Light-yellow	Good	6.77 ± 0.34^a	5096.67 ± 160.35^d
RT	Good	No	Light-yellow	Good	6.96 ± 0.05^a	5603.00 ± 144.00^c
LT	Good	No	Light-yellow	Good	6.93 ± 0.04^a	4919.00 ± 98.88^d
HT	Good	No	Light-yellow	Good	6.88 ± 0.09^a	5811.00 ± 92.24^b
H/C cycles	Good	No	Light-yellow	Good	6.95 ± 0.03^a	5870.00 ± 81.00^a

Values are expressed as mean $\pm$ standard deviation (SD) of triplicate experiments ($n = 3$). Within each column, values labeled with distinct superscript letters are significantly different ($p < 0.05$) compared with the initial condition.

The findings indicated that both the cream base formula (data not shown) and the cream containing RD43 brown rice extract showed good stability and satisfactory physical properties across the storage conditions evaluated, compared with the initial state. The parameters of homogeneity, phase separation, skin feel, and color remained consistent, similar to the pH value. Notably, the viscosity remained stable ($p > 0.05$) throughout the storage period at $4 \pm 1^\circ\text{C}$, indicating no significant structural alterations at lower temperatures. Although the cream formulation did not exhibit visible phase separation, creaming, or cracking during storage, statistically significant changes in viscosity were observed under certain storage conditions. Viscosity variations are commonly used as sensitive indicators of physical changes in emulsion systems and may reflect altered droplet interactions, partial flocculation, coalescence, or temperature-induced changes in the continuous phase. Elevated temperatures and repeated freeze–thaw or heating–cooling cycles can influence emulsion microstructure by affecting droplet size distribution, interfacial film integrity, and the rheological properties of the continuous phase [44]. Despite the observed viscosity changes, no macroscopic instability, such as phase separation, creaming, or cracking, was detected during storage. This suggests that the emulsion system maintained its overall structural integrity under the tested conditions. However, the viscosity variations indicate that temperature-dependent structural modifications may have occurred within the formulation. Similar observations have been reported in cosmetic emulsions, where significant viscosity changes were detected without visible emulsion breakdown during short-term stability testing [45]. This suggests that the cream with the extract maintained good physicochemical stability during storage at low temperatures. In contrast, the other tested conditions showed a significant difference in viscosity ($p < 0.05$) but did not stratify. A limitation of this study is that the stability assessment was conducted over a relatively short period of 30 days. Therefore, the observed physicochemical stability should be considered preliminary and indicative only of short-term formulation performance. Although no significant changes in appearance, pH, viscosity, or phase separation were observed during the study period, these findings are insufficient to confirm long-term product stability or shelf-life. Further real-time and accelerated stability studies conducted over extended storage periods are necessary to establish the long-term stability and commercial applicability of the formulation.

4. Conclusions

In the present study, the 70% ethanolic extract from RD43 brown rice exhibited free radical scavenging properties associated with antioxidants such as phenolic acids, flavonoids, and gamma-oryzanol. The RD43 brown rice extract showed no toxic effects on normal human dermal fibroblast (NHDF) cells. Although the extract demonstrated favorable cytocompatibility toward NHDF cells, the present study did not evaluate skin irritation potential. Cell viability assays provide information regarding cellular toxicity but do not directly assess irritation responses in skin tissue. Therefore, the safety data generated in this study should be considered preliminary. Further investigations using validated skin irritation models, such as reconstructed human epidermis (RHE) assays, HET-CAM testing, and ultimately human patch testing, are necessary to

confirm dermal safety and support cosmetic applications of the formulation. Additionally, the anti-inflammatory effects of the RD43 brown rice extract were demonstrated by its ability to inhibit nitric oxide production, an inflammatory mediator, which is beneficial for treating and alleviating inflammatory responses on the skin after sun exposure. The stability and physical properties of the topical emulsion containing RD43 brown rice extract were evaluated. The results showed satisfactory physicochemical stability under all tested conditions compared with the initial state. This research also emphasizes the potential of Thai RD43 brown rice extract as an antioxidant active ingredient for innovative skin cosmetics. The formulation shows potential for cosmetic application; however, further dermal safety and skin irritation studies are required before practical or commercial use can be recommended.

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References

- [1] Kammeyer, A.; Luiten, R. M. Oxidation Events and Skin Aging. *Ageing Res. Rev.* **2015**, *21*, 16–29. <https://doi.org/10.1016/j.arr.2015.01.001>
- [2] Shindo, Y.; Witt, E.; Packer, L. Antioxidant Defense Mechanisms in Murine Epidermis and Dermis and Their Responses to Ultraviolet Light. *J. Invest. Dermatol.* **1993**, *100*, 260–265. <https://doi.org/10.1111/1523-1747.ep12469048>
- [3] Mittal, M.; Siddiqui, M. R.; Tran, K.; Reddy, S. P.; Malik, A. B. Reactive Oxygen Species in Inflammation and Tissue Injury. *Antioxid. Redox Signal.* **2014**, *20*, 1126–1167. <https://doi.org/10.1089/ars.2012.5149>
- [4] Yasui, H.; Sakurai, H. Chemiluminescent Detection and Imaging of Reactive Oxygen Species in Live Mouse Skin Exposed to UVA. *Biochem. Biophys. Res. Commun.* **2000**, *269*, 131–136. <https://doi.org/10.1006/bbrc.2000.2254>
- [5] Subedi, L.; Lee, T. K.; Wahedi, H. M.; Baek, S. H.; Kim, S. Y. Resveratrol-Enriched Rice Attenuates UVB-ROS-Induced Skin Aging via Downregulation of Inflammatory Cascades. *Oxid. Med. Cell. Longev.* **2017**, 8379539. <https://doi.org/10.1155/2017/8379539>
- [6] Lee, M. S.; Oh, Y. J.; Kim, J. W.; Han, K. M.; Kim, D. S.; Park, J. W.; Kim, H. M.; Kim, D. W.; Kim, Y.-S. Antioxidant, Whitening, Antiwrinkle, and Anti-Inflammatory Effect of *Ajuga spectabilis* Nakai Extract. *Plants* **2023**, *12*, 79. <https://doi.org/10.3390/plants12010079>
- [7] Hoang, H. T.; Moon, J.-Y.; Lee, Y.-C. Natural Antioxidants from Plant Extracts in Skincare Cosmetics: Recent Applications, Challenges and Perspectives. *Cosmetics* **2021**, *8*, 106. <https://doi.org/10.3390/cosmetics8040106>
- [8] Tungmunthum, D.; Thongboonyou, A.; Pholboon, A.; Yangsabai, A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines* **2018**, *5*, 93. <https://doi.org/10.3390/medicines5030093>
- [9] Goufo, P.; Trindade, H. Rice Antioxidants: Phenolic Acids, Flavonoids, Anthocyanins, Proanthocyanidins, Tocopherols, Tocotrienols, γ -Oryzanol, and Phytic Acid. *Food Sci. Nutr.* **2014**, *2*, 75–104. <https://doi.org/10.1002/fsn3.86>

- [10] [10] Hansakul, P.; Srisawat, U.; Itharat, A.; Lerdvuthisopon, N. Phenolic and Flavonoid Contents of Thai Rice Extracts and Their Correlation with Antioxidant Activities Using Chemical and Cell Assays. *J. Med. Assoc. Thai.* **2011**, 94(Suppl. 7), S122–S130.
- [11] Teeranachaideekul, V.; Wongrakpanich, A.; Leanpolchareanchai, J.; Thirapanmethee, K.; Sirichaovanichkarn, C. Characterization, Biological Activities and Safety Evaluation of Different Varieties of Thai Pigmented Rice Extracts for Cosmetic Applications. *Pharm. Sci. Asia* **2018**, 45, 140–153. <https://doi.org/10.29090/psa.2018.03.140>
- [12] Thitipramote, N.; Imsonpang, S.; Sukphopetch, P.; Pradmeeteekul, P.; Nimkamnerd, J.; Nantitanon, W.; Chaiyana, W. Health Benefits and Safety of Red Pigmented Rice (*Oryza sativa* L.): In Vitro, Cellular, and In Vivo Activities for Hair Growth Promoting Treatment. *Cosmetics* **2022**, 9, 111. <https://doi.org/10.3390/cosmetics9060111>
- [13] Yakaew, S.; Phimmuan, P.; Tiensomjit, K.; Nakyai, W.; Nuengchamnong, N.; Ross, G.; Ungsurungsei, M.; Viyoch, J. Hom-Kularb-Dang Rice Bran Extract for the Prevention of UVB-Damage against Human Skin Fibroblast. *CMU J. Nat. Sci.* **2020**, 19, 34–51. <https://doi.org/10.12982/CMUJNS.2020.0003>
- [14] Nilkamheang, T. Evaluation of the Glycemic Index of RD43-Rice, and Its Products by Means of In Vitro Glucose Digestion Mimic Techniques. *JFTSU* **2021**, 16, 59–77. (In Thai).
- [15] Yamsaray, M.; Sreewongchai, T.; Phumichai, C.; Chalermchaiwat, P. Yield and Nutritional Properties of Improved Red Pericarp Thai Rice Varieties. *ScienceAsia* **2023**, 49, 155–160. <https://doi.org/10.2306/scienceasia1513-1874.2022.144>
- [16] Okonogi, S.; Kaewpinta, A.; Junmahasathien, T.; Yotsawimonwat, T. Effect of Rice Variety and Modification on Antioxidant and Anti-Inflammatory Activities. *Drug Discov. Ther.* **2018**, 12, 206–213. <https://doi.org/10.5582/ddt.2018.01041>
- [17] Maizura, M.; Aminah, A.; Wan-Aida, W. M. Total Phenolic Content and Antioxidant Activity of Kesum (*Polygonum minus*), Ginger (*Zingiber officinale*) and Turmeric (*Curcuma longa*) Extract. *Int. Food Res. J.* **2011**, 18, 529–534.
- [18] Sulaiman, S. F.; Yusoff, N. A. M.; Eldeen, I. M.; Seow, E. M.; Sajak, A. B.; Ooi, K. L. Correlation between Total Phenolic and Mineral Contents with Antioxidant Activity of Eight Malaysian Bananas (*Musa* sp.). *J. Food Compos. Anal.* **2011**, 24, 1–10. <https://doi.org/10.1016/j.jfca.2010.04.005>
- [19] Bucci, R.; Magri, A. D.; Magri, A. L.; Marini, F. Comparison of Three Spectrophotometric Methods for the Determination of γ -Oryzanol in Rice Bran Oil. *Anal. Bioanal. Chem.* **2003**, 375, 1254–1259. <https://doi.org/10.1007/s00216-002-1700-5>
- [20] Butsat, S.; Siriamornpun, S. Phenolic Acids and Antioxidant Activities in Husk of Different Thai Rice Varieties. *Food Sci. Technol. Int.* **2010**, 16, 329–336. <https://doi.org/10.1177/1082013210366966>
- [21] Zhang, X.; Wu, Z.; Weng, P.; Yang, Y. Analysis of Tea Catechins in Vegetable Oils by High-Performance Liquid Chromatography Combined with Liquid-Liquid Extraction. *Int. J. Food Sci. Technol.* **2015**, 50, 885–891. <https://doi.org/10.1111/ijfs.12726>
- [22] Ala, A. A.; Olotu, B. B.; Ohia, C. M. D. Assessment of Cytotoxicity of Leaf Extracts of *Andrographis paniculata* and *Aspilina africana* on Murine Cells In Vitro. *Arch. Basic Appl. Med.* **2018**, 6, 61–65.
- [23] Suantawee, T.; Wesarachanon, K.; Anantsuphasak, K.; Daenphetploy, T.; Thien-Ngern, S.; Thilavech, T.; Pasukamonset, P.; Ngamukote, S.; Adisakwattana, S. Protein Glycation Inhibitory Activity and Antioxidant Capacity of Clove Extract. *J. Food Sci. Technol.* **2015**, 52, 3843–3850. <https://doi.org/10.1007/s13197-014-1452-1>
- [24] Lim, Y.; Park, J. W.; Kwon, O. K.; Lee, J. W.; Lee, H. S.; Lee, S.; Choi, S.; Li, W.; Jin, H.; Han, S. B.; Ahn, K. S. Anti-Inflammatory Effects of a Methanolic Extract of *Castanea seguinii* Dode in LPS-Induced RAW264.7 Macrophage Cells. *Int. J. Mol. Med.* **2018**, 41, 391–398. <https://doi.org/10.3892/ijmm.2017.3223>
- [25] Kunnaja, P.; Kumsaiyai, W.; Punturee, K.; Siriphun, A.; Chatboonward, T. Inhibition of Nitric Oxide Production and COX-2 Protein Expression in LPS-Stimulated RAW 264.7 Cells by the Hexane Fraction of *Murdannia loriformis*. *J. Assoc. Med. Sci.* **2019**, 52, 104–112.
- [26] Hyun, S. B.; Hyun, C. G. Anti-Inflammatory Effects and Their Correlation with Microbial Community of Shindari, a Traditional Jeju Beverage. *Fermentation* **2020**, 6, 87. <https://doi.org/10.3390/fermentation6030087>

- [27] Zhang, Q. W.; Lin, L. G.; Ye, W. C. Techniques for Extraction and Isolation of Natural Products: A Comprehensive Review. *Chin. Med.* **2018**, *13*, 20. <https://doi.org/10.1186/s13020-018-0177-x>
- [28] Imam, M. U.; Musa, S. N.; Azmi, N. H.; Ismail, M. Effects of White Rice, Brown Rice and Germinated Brown Rice on Antioxidant Status of Type 2 Diabetic Rats. *Int. J. Mol. Sci.* **2012**, *13*, 12952–12969. <https://doi.org/10.3390/ijms131012952>
- [29] Asnaashari, M.; Emami, S. A.; Tayarani-Najaran, Z. The Effect of Hashemi Brown and White Rice Extracts and γ -Oryzanol on Proliferation and Estrogenic Activity Induced by Zearalenone in MCF-7 Cells. *J. Food Meas. Charact.* **2023**, *17*, 1–14. <https://doi.org/10.1007/s11694-022-01769-5>
- [30] Summpunn, P.; Panpipat, W.; Manurakchinakorn, S.; Bhoopong, P.; Cheong, L. Z.; Chaijan, M. Comparative Analysis of Antioxidant Compounds and Antioxidative Properties of Thai Indigenous Rice: Effects of Rice Variety and Processing Condition. *Molecules* **2022**, *27*, 5180. <https://doi.org/10.3390/molecules27165180>
- [31] Juricic, H.; Cuccioloni, M.; Bonfili, L.; Angeletti, M.; Uberti, D.; Eleuteri, A. M.; Abate, G.; Cecarini, V. Biochemical, Biological, and Clinical Properties of γ -Oryzanol. *Antioxidants* **2025**, *14*, 1099. <https://doi.org/10.3390/antiox14091099>
- [32] Cicero, A. F. G.; Gaddi, A. Rice Bran Oil and γ -Oryzanol in the Treatment of Hyperlipoproteinaemias and Other Conditions. *Phytother. Res.* **2001**, *15*, 277–289. <https://doi.org/10.1002/ptr.907>
- [33] de Lima Cherubim, D. J.; Buzanello Martins, C. V.; Oliveira Fariña, L.; da Silva de Lucca, R. A. Polyphenols as Natural Antioxidants in Cosmetics Applications. *J. Cosmet. Dermatol.* **2020**, *19*, 33–37. <https://doi.org/10.1111/jocd.13093>
- [34] Khan, R. A.; Khan, M. R.; Sahreen, S.; Ahmed, M. Evaluation of Phenolic Contents and Antioxidant Activity of Various Solvent Extracts of *Sonchus asper* (L.) Hill. *Chem. Cent. J.* **2012**, *6*, 12. <https://doi.org/10.1186/1752-153X-6-12>
- [35] Lalhminghlui, K.; Jagetia, G. C. Evaluation of the Free-Radical Scavenging and Antioxidant Activities of Chilauni, *Schima wallichii* Korth. In Vitro. *Future Sci. OA* **2018**, *4*, FSO272. <https://doi.org/10.4155/fsoa-2017-0086>
- [36] Sharma, J. N.; Al-Omran, A.; Parvathy, S. S. Role of Nitric Oxide in Inflammatory Diseases. *Inflammopharmacology* **2007**, *15*, 252–259. <https://doi.org/10.1007/s10787-007-0013-x>
- [37] Aktan, F. iNOS-Mediated Nitric Oxide Production and Its Regulation. *Life Sci.* **2004**, *75*, 639–653. <https://doi.org/10.1016/j.lfs.2003.10.042>
- [38] Lambers, H.; Piessens, S.; Bloem, A.; Pronk, H.; Finkel, P. Natural Skin Surface pH Is on Average below 5, Which Is Beneficial for Its Resident Flora. *Int. J. Cosmet. Sci.* **2006**, *28*, 359–370. <https://doi.org/10.1111/j.1467-2494.2006.00344.x>
- [39] Shao, Y.; Xu, F.; Sun, X.; Bao, J.; Beta, T. Identification and Quantification of Phenolic Acids and Anthocyanins in Rice Kernels. *J. Cereal Sci.* **2014**, *59*, 211–218. <https://doi.org/10.1016/j.jcs.2014.01.004>
- [40] Adom, K. K.; Liu, R. H. Antioxidant Activity of Grains. *J. Agric. Food Chem.* **2002**, *50*, 6182–6187. <https://doi.org/10.1021/jf0205099>
- [41] Shahidi, F.; Yeo, J. D. Insoluble-Bound Phenolics in Food. *Molecules* **2016**, *21*, 1216. <https://doi.org/10.3390/molecules21091216>
- [42] Palungwachira, P.; Tancharoen, S.; Phruksaniyom, C.; Klungsaeng, S.; Srichan, R.; Kikuchi, K.; Nararatwanchai, T. Antioxidant and Anti-Inflammatory Properties of Anthocyanins Extracted from *Oryza sativa* L. in Primary Dermal Fibroblasts. *Oxid. Med. Cell. Longev.* **2019**, 2089817. <https://doi.org/10.1155/2019/2089817>
- [43] Zhang, Y.; Lane, M. E.; Moore, D. J. An Investigation of the Influence of PEG 400 and PEG-6-Caprylic/Capric Glycerides on Dermal Delivery of Niacinamide. *Polymers* **2020**, *12*, 2907. <https://doi.org/10.3390/polym12122907>
- [44] Tadros, T. F. *Emulsion Formation and Stability*; Wiley-VCH: Weinheim, Germany, 2013. <https://doi.org/10.1002/9783527647941>
- [45] Barel, A. O.; Paye, M.; Maibach, H. I., Eds. *Handbook of Cosmetic Science and Technology*, 4th ed.; CRC Press: Boca Raton, FL, **2014**. <https://doi.org/10.1201/b16716>