"Red Rice Extract as a Biological UV filter and Its Photo-protective Enhancement Effects"

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1. Introduction

Solar UV radiation is typically divided into three bands based on its wavelength: UVC (100–280 nm), UVB (280–320 nm), and UVA (320–400 nm). While UVC radiation is almost completely absorbed by the ozone layer, UVA and some UVB radiation can penetrate the atmosphere [1]. The prolonged exposure of skin to such UV radiation can lead to a variety of harmful effects, including immune suppression, photoaging, and carcinogenesis. This can trigger several skin conditions, such as sunburn, pigmentation disorders, actinic keratosis, and skin cancers [2]. Sunscreen products are effective at mitigating the harmful effects of UV damage, thus preventing the onset of such skin conditions.

Current sunscreen products typically contain either chemical or physical UV filters that protect the skin from UV damage [3]. Physical UV filters can effectively reflect or scatter solar UV radiation, but their high concentrations in sunscreen formulations can produce a white cast and lead to a poor sensory feel on the skin. Chemical UV filters, while effective, can pose potential toxicological risks and have been linked to skin allergies and phototoxicity. Overuse of these chemical UV filters in sunscreen formulations can also result in an unpleasant, greasy skin feel. Additionally, there are some environmental concerns, including coral bleaching, associated with the use of these UV filters [3,4]. In contrast, biological UV filters offer dual benefits: they not only absorb UV radiation but also provide antioxidant, anti-aging, and whitening effects [5,6]. These filters generally have a low irritation potential and are environmentally friendly and sustainable. As a result, research on biological UV filters is increasing to promote their incorporation into sunscreen formulations and enhance photoprotective efficacy while producing mild and safe products with a pleasant skin feel [7,8].

Red rice, a variety of pigmented rice, has garnered attention due to its rich content of bioactive compounds. Research has demonstrated that red rice contains higher total phenolic and flavonoid levels than other rice varieties, such as black, purple, and white rice [9]. The primary phenolic acids found in red rice include ferulic acid, ρ-coumaric acid, isoferulic acid, syringic acid, and caffeic acid. Additionally, red rice is rich in proanthocyanidins and anthocyanidins, which contribute to its superior antioxidant capacity relative to other rice varieties [10]. Notably, ferulic acid, when combined with conventional UV filters, has been shown to increase SPF values by as much as 37% [11]. Proanthocyanidins have also been studied for their potential

application in sunscreen products, and camphor ethanol extracts rich in proanthocyanidins have been shown to exhibit higher SPF values than acetone and homosalate extracts [12]. Song and colleagues utilized oligomeric proanthocyanidins to construct composite microcapsules, which improved the stability of the UVB filter octyl methoxycinnamate (OMC) and broadened its protective range to include UVA radiation [13].
Although red rice contains several UV-protective components, research on its photoprotective properties remains limited. A study by Limtrakul et al. demonstrated that red rice extract mitigates UVB-induced inflammation and skin aging by inhibiting the MAPK signaling pathway [14]. Similarly, Zhang et al. showed that red rice extract can protect against blue light (BL)-induced cellular damage by reducing reactive oxygen species (ROS) production in HaCat cells, inhibiting melanin synthesis in B16 cells, and enhancing COL1A1 gene expression in human fibroblast cells [6]. Additionally, Hom-Kularb-Dang rice bran extract has been found to protect human dermal fibroblasts from UVB-induced photoaging by decreasing matrix metalloproteinase-1 (MMP-1) expression and promoting type I procollagen production [5]. Zhang et al. have summarized the polyphenols, flavonoids, and related compounds present in pigmented rice bran such as red rice, along with their photoprotective and anti-photoaging mechanisms [15]. These studies confirm the photoprotective effects of red rice extract at the cellular level. However, comprehensive investigations into its UV absorption spectrum and potential as a photoprotective enhancer in sunscreen formulatins are currently lacking.
This study evaluated the potential of red rice extract as a biological UV filter through UV absorption curve analysis and photothermal stability tests. Furthermore, the extract was incorporated into various sunscreen formulations, and SPF values were measured to assess its ability to enhance SPF performance and serve as a potential substitute for chemical UV filters. The aim of this study was to explore the feasibility of using red rice extract as a natural alternative in sunscreen products.

2. Materials and Methods

2.1 Materials, reagents, and instruments

Liquid Red Rice Extract: The liquid red rice extract was prepared by mixing 100 g of red rice bran powder with 500 g of a 70% butylene glycol aqueous solution. The mixture was stirred at 40°C for 12 h, centrifuged to collect the supernatant, and filtered through a 0.50 μm membrane to obtain the liquid red rice extract. The specific preparation method was based on prior research [16].
The following reagents were used: Ethanol (AR, Shanghai Titan Scientific Co., Ltd); Dimethyl sulfoxide (DMSO; AR, Sinopharm Chemical Reagent Co., Ltd); Ethylhexyl salicylate, octocrylene, and methylene bis-benzotriazolyl tetramethylbutylphenol (MBBT), sourced from Tianjin Aiqishi Technology Co., Ltd; and gallic acid (99%+), purchased from Shanghai Aladdin BioChem Technology Co., Ltd; Green tea extract, Ingredi Biotechnology Co., Ltd.
The instruments used were as follows: Ultrospec 2100 pro UV-visible spectrophotometer from General Electric Company; Labsphere UV2000S UV transmittance analyzer from Shanghai Lanfei Optical Equipment Co., Ltd; Forced-air drying oven from Shanghai Bluepard Instruments Co., Ltd;

2.2 Experimental Methods

2.2.1 UV absorption curve analysis

The liquid red rice extract was diluted with DMSO to obtain actives concentration of 100 ppm, 300 ppm, and 500 ppm. Traditional UV filters (ethylhexyl salicylate, octocrylene and MBBT) were diluted to a concentration of 100 ppm for comparison. The absorbance curves of these solutions were obtained over the wavelength range of 280–500 nm using a UV-visible spectrophotometer. The area under the curve (AUC) was quantified through numerical integration using GraphPad Prism version 9.0.0 for Windows (San Diego, California USA).

2.2.2 DPPH radical scavenging assay

The DPPH radical scavenging rate of the red rice extract was tested according to the group standard T/SHRH 006-2018: Cosmetics—Free Radical (DPPH) Scavenging Experiment Method. [17]

2.2.3 Preparation of sunscreen formulations containing red rice extract

Different amounts of red rice extract were added to various sunscreen formulations, and water was used to adjust the total volume to 100%. The specific formulations are listed in **Table 1**.

Table 1. Formulations and Proportions of Components in Each Formulation

Phase	Component		Formula 1 (%)	Formula 2 (%)	Formula 3 (%)	Formula 4 (%)	Formula 5 (%)	Formula 6 (%)	Formula 7 (%)
A	Water		Adjust to 100%						
	Chelating Agent		0.10	0.10	0.02	0.02	0.05	0.02	0.00
	Humectant		4.00	3.00	5.00	6.00	3.00	3.00	10.00
	Thickener		0.44	0.80	0.87	0.60	0.05	2.20	5.00
	Skin Conditioning Agent		1.40	0.80	0.00	0.00	0.28	0.28	3.00
B	UV filters	Large Molecule UV filters	2.00	11.00	1.50	6.00	4.00	0.00	2.50
		Small Molecule UV filters	15.00	3.50	21.00	12.00	12.50	0.00	12.00
		Physical UV filters	0.00	0.00	0.00	3.00	8.00	20.00	8.00
	Emollient		1.30	8.00	11.71	11.20	34.50	26.20	20.00
	Oil Phase Film Former		1.13	0.50	0.00	0.00	0.00	0.00	13.50
	Emulsifier		2.00	1.85	1.75	3.18	1.40	5.00	4.50
C	pH Adjuster		0.15	0.00	1.15	0.18	1.70	0.00	2.00
	Sensory Modifier		1.00	0.00	1.00	3.50	11.00	3.50	1.00
	Preservative		0.00	0.00	0.80	0.80	1.00	0.80	0.00
	Red Rice Extract		0.00-5.00						

The preparation protocol is described as follows:

Oil-in-Water (O/W) System: 1). The raw materials in phases A and B each were mixed and heated separately to 80°C. The mixtures were stirred until a homogenous solution was obtained; 2). Phase B was added to phase A under high-temperature homogenization (7 min, 5000 rpm); 3). Phase C, which contained red rice extract, was added to the mixture. The mixture was stirred and cooled to room temperature, and the volume was adjusted to 100% using water.

Water-in-Oil (W/O) System: 1). The solids and liquids of phase A were heated and dissolved before cooling to room temperature and adding the remaining ingredients of phase A; 2). The solids and liquids of phase B were heated and dissolved before cooling to room temperature

and adding the remaining ingredients of phase B; 3) Phase B was slowly added to phase A, and this was followed by emulsification and homogenization; 3). Phase C was added to the AB mixture and homogenized thoroughly. The detailed formulations are shown in **Table 1**.
The following formula was applied to calculate the UV filters percentage increase:
X%=(Amount of UV filters Added/Total UV filters Amount)*100%

2.2.4 SPF value determination

A 0.0325 g sample of glycerin was evenly applied onto a textured polymethyl methacrylate (PMMA) plate for a blank scan. Then, 0.0325 g of the sample was evenly applied to the PMMA plate using a syringe. During testing, the researchers wore disposable latex gloves, and the sample was spread evenly on the plate by applying approximately 200 g of pressure with a fingertip. After the sample was allowed to dry and form a film in the dark for 20 min, the UV transmission was measured using the Labsphere UV2000S UV transmittance analyzer. The SPF value was calculated using computer software UV2000S Application. The experiment should be conducted under the following conditions: temperature of 25°C ± 5°C, humidity of 50% ± 10%, and in a dark environment. Each sample was tested in triplicate, and the average value as the experimental results.[18]

2.2.5 Statistical analysis

All curves and bar charts were generated using GraphPad Prism version 9.0.0 for Windows (San Diego, California USA). All statistical analyses were performed with Graphpad Prism 9.0.0 for Windows (San Diego, California USA). Quantitative data accord with normal distribution,with mean ± standard deviation. For two-group comparison, P values were derived from the one-way Student t test to determine differences between groups. A value of $P < 0.05$ was considered significant.

3. Results

3.1 Comparison of UV protection performance between red rice extract and Traditional UV filters

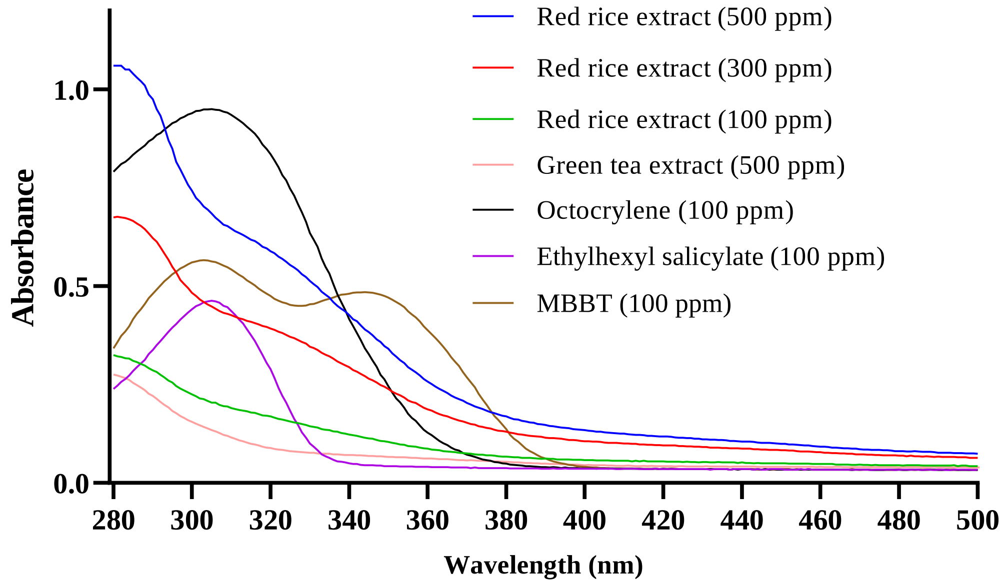

Figure 1. UV Absorption Spectra of Red Rice Extract, Octocrylene, Ethylhexyl Salicylate, MBBT and Green tea extract.

Ethylhexyl salicylate, octocrylene and MBBT are all UV filters permitted for use in cosmetics according to the China Cosmetics Safety Technical Specifications (2015 edition). Ethylhexyl salicylate primarily provides UVB protection, octocrylene offers good protection in the UVB and UVA2 (320–340 nm) ranges, and MBBT provides UV protection across both the UVA and UVB bands. To assess the UV protection performance of red rice extract, this study compared its UV absorption spectrum with that of the three aforementioned chemical UV filters. To ensure complete sample dissolution and eliminate the effects of different solvents, DMSO was used as the solvent for all tested samples. Red rice extract was diluted to 500, 300, and 100 ppm, while the three chemical UV filters were diluted to 100 ppm. The UV absorption spectra were measured in the range of 280–500 nm.

Table 2. Peak Area of Red Rice Extract, Octocrylene, Ethylhexyl Salicylate MBBT and green tea extract under Different Wavelength Ranges (Unit: AU·nm)

Wave-length Range (nm)	Red rice extract (500 ppm)	Red rice extract (300 ppm)	Red rice extract (100 ppm)	Octo-crylene (100 ppm)	Ethylhexyl salicylate (100 ppm)	MBBT (100 ppm)	Green tea extract (500 ppm)
280-320	30.50	19.32	8.11	34.44	13.65	18.7	5.35
320-400	20.78	13.97	4.80	16.71	1.89	21.89	2.18
400-500	6.55	4.84	1.53	0.00	0.00	0.04	0.69

The experimental results (**Figure 1**) showed that red rice extract exhibited absorption intensity. The absorbance was particularly strong in the 280-320 nm range. Moreover, red rice extract also demonstrated the absorption in the UVA (320-400nm) and the BL range (400-500 nm). Integrated peak areas were quantified using GraphPad Prism (v9.0.0) and results are summarized in **Table 2**. These data indicates red rice extract has excellent absorption capacity in UVB and UVA range. Furthermore, red rice extract has certain absorption in BL range that octocrylene, ethyhexyl salicylate and MBBT lacked.

To compare with known biological UV filters, the absorbance of green tea extract was tested, and red rice extract exhibited significantly stronger UV absorption compare to green tea extract at the same concentration. These findings suggest red rice extract may possess photoprotective potential as a biological UV filter. Furthermore, biological UV filters demonstrate dual photoprotective mechanisms: not only through direct UV absorption, but also via antioxidant protection.

3.2 DPPH scavenging capability of red rice extract

Plant extracts, due to their content of flavonoids, phenols, and other compounds, not only provide UV protection but can also inhibit melanin production [18]. The synthesis of melanin is closely related to the generation of oxidative free adicals. Therefore, while evaluating the potential of red rice extract as a biological UV filter, it is also important to assess its antioxidant properties. In the tested concentration range, the DPPH scavenging rate of red rice extract was found to be linearly correlated to its concentration (**Figure 2**). The EC_{50} (concentration at which 50% scavenging is achieved) of red rice extract was 68.32 ppm, with the positive control Vitamin E (Ve) exhibited the EC_{50} of 28.18 ppm. Meanwhile, the DPPH scavenging rate of green tea extract was tested, which showed an EC_{50} value of 193.20 ppm, whereas red rice extract demonstrated significantly DPPH radical scavenging capacity compared to green tea extract.

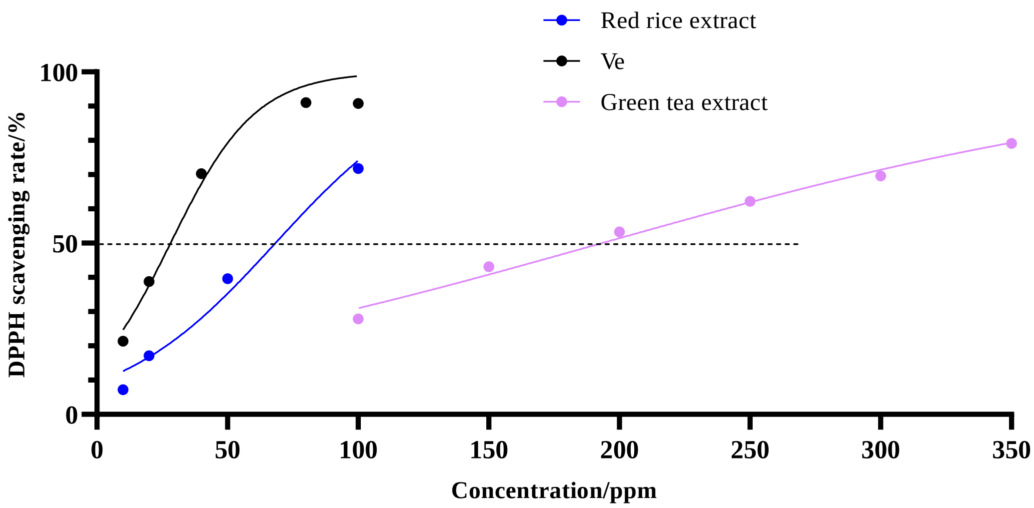

Figure 2. DPPH Scavenging Activities of Red Rice Extract Green tea extract and Vitamin E

3.3 SPF enhancement effect of different red rice extract concentrations

Red rice extract was added to the oil-in-water system of Formula 1 at concentrations of 1%, 3%, and 5% to study the influence of extract concentration on the enhancement of SPF values. The SPF values of both the base formulation and the formulations containing added red rice extract were examined. As shown in **Table 3**, the SPF values increased with the amount of red rice extract added. Moreover, the addition of even 1% red rice extract resulted in a significant SPF enhancement of 14.7%. These findings demonstrated that 1% red rice extract offers a notable SPF enhancement effect. Therefore, the 1% concentration was selected for further experiments using different formulations.

Table 3. SPF Values of Formula 1 Supplemented with Different Concentrations of Red Rice Extract

Sample	Formula 1	Formula 1 + Red Rice Extract		
Red Rice Extract Concentration (%)	-	1%	3%	5%
SPF Value	43.93 ± 1.73	50.38 ± 3.75	52.69 ± 0.28	55.41 ± 1.42
SPF Enhancement (%)	-	14.7	19.9	26.1

3.4 SPF enhancement effect of red rice extract in different formulation systems

Based on their mode of emulsification, sunscreen products are categorized into W/O and O/W systems. Furthermore, based on the mechanisms of sun protection, the formulations can further be classified into physical, chemical, and physical-chemical hybrid UV filters' systems. According to the regulations of the National Medical Products Administration, the transdermal absorption rate is significantly reduced for UV filters with a molecular weight greater than 500 Da. Therefore, UV filters are also classified based on the size of the molecules (molecular weight above or below 500 Da), i.e., those primarily containing large-molecule UV filters and those primarily containing small-molecule UV filters.

Table 4. SPF Values of Different Sunscreen Formulations Supplemented with Red Rice Extract

System		Formulation No.	SPF of Base Formula	SPF After Adding 1% Red Rice Extract	SPF Enhancement (%)
O/W	Mainly based on Large-molecule UV filters	Formula 2	33.17± 6.48	38.92 ± 8.73	17.3%
	Mainly based on small-molecule UV filters	Formula 3	38.05 ± 1.64	43.96 ± 1.70	15.5%
	Physical-chemical combined UV filters	Formula 4	35.37 ± 3.63	40.25 ± 3.14	13.8%
W/O	Physical-chemical combined UV filters	Formula 5	69.48 ± 3.41	78.90 ± 5.41	13.6%
	Physical UV filters	Formula 6	59.92 ± 11.76	66.59 ± 11.06	11.1%
W/O foundation	Physical-chemical combined UV filters	Formula 7	69.28 ± 3.87	89.02 ± 3.40	28.5%

To examine the SPF enhancement effect of red rice extract in different systems, this study selected six different sunscreen formulations and added 1% red rice extract to each of them. The SPF values before and after adding red rice extract were tested to measure the photoprotective enhancement effect (**Table 4**). After adding 1% red rice extract, the SPF enhancement effect in all formulations exceeded 10%. These findings suggested the presence of synergistic effects between certain UV filters and red rice extract.

3.5 Substitution of traditional UV filters with red rice extract equivalents

Table 5. SPF Values of Formula 1 Supplemented with Red Rice Extract Instead of Traditional UV Filters

Sample	Formula 1			Formula 1 + Red Rice Extract		Formula 1 + Tranditional UV filters	
Red Rice Extract (%)	0.00	1.00	3.00	5.00	0.00	0.00	0.00
UV filters (%)	17.00	17.00	17.00	17.00	19.75	21.00	23.00
SPF Value	43.93 ± 1.73	50.38 ± 3.75	52.69 ± 0.28	55.41 ±1.42	50.62±1.06	52.95 ±0.78	54.17 ±1.24
SPF inhancement (%)	0.00	1.00	3.00	5.00	0.00	0.00	0.00

Biological UV filters offer significant advantages over traditional UV filters. They are not harmful to the environment, and they are biodegradable, eco-friendly, and sustainable. Moreover, they show low toxicity and produce minimal irritation on the human body. Therefore, biological UV filters can partially or completely replace traditional UV filters, reducing potential harms to both humans and the environment. To this end, we explored the potential of red rice extract as a substitute for traditional UV filters to guide its practical applications.

An O/W sunscreen emulsion with an SPF value of 43.93 ± 1.73 (Formula 1) was selected, and the effect of adding red rice extract instead of the original UV filters was examined. The goal was to identify what proportion of UV filters could be replaced by red rice extract while maintaining the SPF value. As shown in **Table 5**, the SPF values of the formulations containing 17.00% UV filters + 1.00%, 17.00% UV filters + 3.00%, and 17.00% UV filters + 5.00% red rice extract were similar to those of formulations containing 19.75%, 21.00%, and 23.00% of traditional UV filters.
Accordingly, the calculations showed that 1.00%, 3.00%, and 5.00% red rice extract can replace approximately 12.82%, 19.05%, and 26.09% of the total UV filters in the original formula. This indicated that red rice extract could serve as a good substitute for traditional UV filters. Incorporating red rice extract into sunscreen products may thus effectively reduce the amount of traditional UV filters required, improve safety, reduce skin irritation, enhance the sensory experience, and mitigate the environmental impact of chemical UV filters.

4. Discussion

Red rice extract demonstrates promising broad-spectrum UV protection capabilities, effectively absorbing UVA, UVB and BL. This represents a critical advantage over traditional chemdical UV filters, which typically lack BL absorption capacity. Additionally, red rice extract exhibited antioxidant activity with IC50 values of 68.32 ppm using DPPH assay, which is consistant with the research by W. Sinthorn et al [9].
Further investigation into its photoprotective enhancing effects demonstrated that it can enhance the SPF value of sunscreen formulations and products. Results showed that adding 1% red rice extract increased the SPF of various sunscreen formulations by more than 10%. In addition, substitution experiments showed that 1.00%, 3.00%, and 5.00% of red rice extract could replace approximately 12.82%, 19.05%, and 26.09% of the total amount of traditional UV filters present in the formulations, respectively.
However, this study did not investigate the bioactive compounds responsible for the photoprotective effects in red rice extract and the mechanisms underlying of SPF enhancement effect in sunscreen formulations were not entirely delineated. Further analysis should isolate active compounds and explore its synergistic effects with other UV filters and investigate the specific mechanisms of its SPF enhancement properties in different systems..

5. Conclusion

Biological UV filters offer several advantages, including minimal skin irritation, renewability, and environmental friendliness. The substitution of traditional UV filters with biological alternatives is among the most popular trends in sunscreen development today. This study establishes red rice extract has the following ablities as a multifunctional biolocial UV filters: (1) Broad-spectrum UV protection capabilities, addressing a critical gap in traditional UV filters; (2) Effective SPF enhancement, adding 1% red rice extract can enhance over more 10% SPF; (3) Replacement of traditional UV filters, 5% red rice extract can relace 26.09% traditional UV filters in the sunscreen formulations. These findings demonstrate the photoprotective capacity and SPF-enhancing efficacy of red rice extract, and provide foundational insights for future studies on biological UV filters.

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