Development of Sunscreen Cream from Riceberry Extract

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Abstract

Riceberry (*Oryza sativa*) is rich in various vitamins and contains an important substance called anthocyanin, which is a highly effective antioxidant. It can inhibit skin damage caused by the oxidative process triggered by ultraviolet light. This research investigated the biological activities of riceberry extract (OSE) and developed a sunscreen formula incorporating this extract. The study assessed the inhibition of tyrosinase enzyme production in melanoma cells (B16F10) and evaluated the properties and stability of the product under accelerated conditions. It was determined that riceberry extract (OSE) at concentrations ranging from 0.0001 to 1 mg/ml was non-toxic to B16F10 cells. At a concentration of 1 mg/ml, riceberry extract (OSE) inhibited melanin production in B16F10 cells by 20.60±3.67% but did not affect the production of the tyrosinase enzyme when compared to kojic acid. Additionally, among six tested sunscreen formulas, the fifth formula proved to be the most stable under accelerated conditions.

Keywords: sunscreen, cosmetic products, riceberry, *Oryza sativa*, melanoma cells (B16F10)

1. Introduction

In Sing Buri province, farmers have formed a group to cultivate chemical-free rice, including the popular jasmine and glutinous rice varieties, alongside a small amount of the trendier Riceberry. Despite the smaller proportion of Riceberry in their crop, there is an increasing trend towards consuming this variety among health-conscious consumers. The group cultivates about 1,500 rai of organic rice as part of the "Safe Rice Producer Network of Sing Buri" initiative. However, a major issue is that Riceberry yields do not command a high price as it is grown in other provinces. Additionally, the leftover Riceberry husks and bran are underutilized. To address this, researchers propose adding value to the rice by producing health-related products, such as cosmetics and traditional Thai medicine items for spa businesses. This would provide additional income for the grassroots-level economy in the community. Researchers are collaborating with a community enterprise group in Bang Rachan district, Sing Buri province, which has potential in producing health products and various crafts.

Red rice and purple rice have a high concentration of combined phenols and flavonoids, as well as significant antioxidant capabilities compared to light-colored rice or other grains (B., Gu, L. et al., 2012). In red rice, it has been found to contain up to 752.1 mg/100g of

polyphenols, 250.36 mg/100g of anthocyanins, and 63.3 ug/100g of beta carotene. It also contains important components such as beta-carotene and lutein, which have been shown to reduce the incidence of colorectal and breast cancer (S., Leardkamolkarn et al., 2016). Anthocyanins from red rice can inhibit the growth and aggregation of calcium oxalate crystals, which may prevent kidney stone formation in experimental tubes (Khawsuk W et al., 2018).

In addition to cancer prevention, studies have shown that supplementation with red rice (up to 41% by weight) in diabetic mice fed a high-fat diet can reduce high blood sugar levels, high blood fat levels, oxidative stress, and inflammation. These effects are believed to be a result of increased levels of antioxidants, improved insulin tolerance, and decreased beta cell death.[3] Furthermore, the use of red rice bran oil (RBBO) helps increase high-density lipoprotein (HDL or good cholesterol) levels and decrease low-density lipoprotein (LDL or bad cholesterol) levels in diabetic mice. Specifically, after 12 weeks of RBBO supplementation, there were significant reductions in malondialdehyde levels and restoration of superoxide dismutase, catalase, glutathione peroxidase, coenzyme Q10, and oxygen radical absorbance capacity (ORAC) levels in diabetic mice (P., Surasiang et al., 2013).

ANT derived from Oryza sativa L. was shown to increase mRNA expression and protein levels of collagen type I alpha 2 (COL1A2) in H2O2-stimulated rat dermal fibroblasts (RDFs) without causing cytotoxic effects. Treatment with ANT activated key signaling pathways such as ERK1/2 and Akt, and significantly reduced the phosphorylation of IκBα and the activation of NF-κB subunits p50 and p65, which are associated with inflammation. These findings suggest that ANT has anti-inflammatory and anti-aging potential by modulating type I collagen expression and inhibiting NF-κB activation in response to oxidative stress (Palungwachira, P et al., 2019).

The cytotoxicity of various rice extracts was assessed using human fibroblast cells to determine their safety for topical use. At a concentration of 400 µg/mL, all rice extracts demonstrated cell viability above 50%, indicating their safety. Among the extracts tested, LP exhibited the highest cell viability compared to others. These findings suggest that rice extracts are promising candidates as cosmetic ingredients, particularly for anti-aging applications (Teeranachaideekul, V et al., 2021).

Conclusion: The effect of various species of rice (Oryza sativa L.) extracts had the high antioxidant, and it was able to stimulate the melanogenesis activity, providing the increase in potential value added of Thai rice with different species. It could beneficially apply for hair treatment formulation in cosmetic products (Soradech, S., 2016).

Therefore, the research team proposed the idea of utilizing the leftover Riceberry residue and bran to develop value-added health products, Cosmetics, such as sunscreen creams, help protect the skin from ultraviolet radiation (UV) damage, including both UVA and UVB rays. They prevent the skin from getting burned or developing dark spots caused by sun exposure, reducing the risk of skin cancer (Phopaporn Srisupap, M.D. (n.d.)).

2. Materials and Methods

Plant material and extraction

The process involves sourcing and cleaning the Riceberry from natural sources and drying them in an oven at 50°C. Weighing according to the specified amount, they are then roughly ground using a disk mill, and macerated with 95% ethanol for 3 days. The mixture is filtered through filter paper, and the residue is re-macerated and filtered twice more. The resulting extracts are combined and concentrated using a rotary evaporator. The percentage yield (%Yield) is calculated.

The formula for calculating the % yield of plant extract (Phrompittayarat et al., 2007) is to compare the amount of extract obtained with the initial amount of plant material used, to determine the production cost for each extraction.

% yield = weight of plant extract / dry weight of plant material × 100

Cell Culture

The B16F10 melanoma cells were cultured and maintained in Dulbecco's Modified Eagle's Medium (DMEM high glucose, Gibco) supplemented with 10% (v/v) Fetal Bovine Serum and 1% antibiotics penicillin/ streptomycin (pen/strep). Maintained at 37°C in a humidified incubator with 5% CO2. The cells were subcultured after 5–6 days when they reached > 80% confluence.

Cytotoxicity Assay

The potential cytotoxicity of Riceberry extract to B16F10 melanoma cells was evaluated by using sulforhodamine B (SRB) assay (Vichai & Kirtikara, 2006). B16F10 cells (1×10^4 cells/well) were incubated in 96-well plates. After treatment with various concentrations (0.0001, 0.001, 0.01, 0.1 and 1 mg/ml) of Riceberry extract, the cells were incubated at 37 °C for 24 h. cell monolayers are fixed with 10% (wt/vol) trichloroacetic acid and stained for 30 min, after which the excess dye is removed by washing repeatedly with 1% (vol/vol) acetic acid. The protein-bound dye is dissolved in 10 mM Tris base solution for OD determination at 510 nm using a microplate reader.

Tyrosinase Activity Assay

Tyrosinase activity was determined by assessing the rate of L-DOPA oxidation, employing a previously described methodology. B16F10 cells (1×10^4 cells/well) were incubated in 24-well plates. Briefly, cells were lysed using a buffer containing 50 mM Tris-HCl (pH 7.0), 2% β-mercaptoethanol, 10 mM EDTA, and a protease inhibitor cocktail. The cell lysates were centrifuged at 12,000 rpm for 30 minutes at 4 ℃ to separate the supernatant. The collected supernatant was subsequently analyzed for tyrosinase activity. The reaction mixture consisted of 0.1 M sodium phosphate (pH 7.0) mixed with an equal volume of 1 mg/mL L-DOPA. Following a 2-hour incubation at 37 ℃, the formation of dopachrome was detected by measuring the absorbance at 405 nm using an ELISA reader. Results were expressed as the percentage change relative to the control. (Manosroi et al., 2013, Su et al., 2013).

Cellular Melanin Content Determination

The melanin content was quantified using a method previously described in the literature. B16F10 cells (1×10^5 cells/well) were incubated in 24-well plates. Briefly, B16F10 cells were treated with Riceberry extract (OS) for 24 h. The cells were washed with PBS and then dissolved in 200 μL of 1 N NaOH at 80 °C for 2 h. The samples were centrifuged for 30 min at 12,000 rpm to collect the supernatant. The relative melanin content was determined by measuring the absorbance at 475 nm (Manosroi et al., 2013, Su et al., 2013).

Safety and physical stability testing of a product (Asawachaisuphon and Chuaiphrom, 2010)

Physical stability assessment by observing the characteristics of the gel texture, phase separation, and odor. pH testing was conducted using a pH meter on the product immediately after preparation and after being stored at room temperature for one week.

Accelerated stability testing was performed by subjecting the product to five freeze-and-thaw cycles. The product was stored in a controlled temperature and humidity cabinet at 4°C for 24 hours before being exposed to a temperature of 45°C for 24 hours, which was considered one cycle. The testing was conducted for five cycles. The stability of the product was evaluated based on the results of this testing.

Development of a formulation of sunscreen cream from Riceberry extract

The physical stability of the product was also evaluated by observing the appearance of the gel, the separation of layers, and the pH of the product when freshly prepared and after one week of storage at room temperature.

In summary, the product derived from the extract of Riceberry extract was evaluated for cytotoxicity potential using in vitro methods. The physical stability of the product was also evaluated.

Table 1: Table description

Ingredients	Amount %w/w					
	1	2	3	4	5	6
Titanium Dioxide 15nm Liquid	10	10	10	10	10	10
Zinc Oxide 35nm Liquid	10	10	10	10	10	10
Silicone Blender	1	2	3	3	4	5
Ethylhexylglycerin (Mild Preserve)	1	1	1	1	1	1
Silicone Gel	26	27	28	28	29	30
D.I. Water	46.75	44.75	42.75	47.75	40.75	38.75
Beige Iron Oxides EasyMix™	0.15	0.15	0.15	0.15	0.15	0.15
Fragrance	0.1	0.1	0.1	0.1	0.1	0.1
Riceberry extract	5	5	5	0	5	5

3. Results

Effect of Cytotoxicity on melanoma cells (B16F10)

Table 2 : Cytotoxicity of Riceberry extract on melanoma cells (B16F10)

Sample / Concentration (mg/ml)	percentage of cell survival				
	0.0001	**0.001**	**0.01**	**0.1**	**1**
Riceberry extract	102.39 ±0.74	101.15 ±1.47	100.09 ±1.09	98.08 ±1.43	96.29 ±2.59

The values represent the mean ± SD for 4 experiments.

The results of Cytotoxicity testing found that the extracts from Riceberry extract at concentrations of 0.0001-1 mg/ml were not toxic to melanoma cells (B16F10). The concentration of 1 mg/ml showed cell survival rates of 96.29±2.59% respectively.

Effect of Tyrosinase Activity in melanoma cells (B16F10)

Table 3 : showing Effect of Tyrosinase inhibition.

Sample	Tyrosinase inhibition %	Potency (equivalent to kojer acid)
Riceberry extract	-53.04±6.89	-
Kojic acid	83.52±3.03	-

The values represent the mean ± SD for 3 experiments.

At a concentration of 1 mg/ml, the extract from Riceberry showed an inhibition of tyrosinase activity in B16F10 melanoma cells by -53.04±6.89%, indicating an increase rather than a decrease in enzyme activity, as negative values typically denote stimulation. In contrast, kojic acid at the same concentration exhibited a substantial inhibition rate of 83.52±3.03%. Therefore, it is evident that the Riceberry extract did not inhibit, but instead potentially stimulated, tyrosinase enzyme activity in B16F10 cells.

Table 4 : showing melanin pigment inhibition test.

Sample	Inhibition of Melanin Pigmentation %	Potency (equivalent to kojer acid)
Riceberry extract	20.60±3.67	0.36
Kojic acid	56.80±3.64	-

The values represent the mean ± SD for 3 experiments.

At a concentration of 1 mg/ml, the extract from Riceberry inhibited melanin synthesis in B16F10 melanoma cells by 20.60±3.67%, whereas kojic acid exhibited a higher inhibition rate of 56.80±3.64% at the same concentration. Therefore, the Riceberry extract demonstrated approximately 0.36 times the inhibition effect on melanin synthesis in B16F10 cells compared to kojic acid.

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Table 5 : shows the experimental results of all 6 formulas.

	1	**2**	**3**	**4**	**5**	**6**
color	bright white	bright white	bright white	bright white	bright white	bright white
smell	Riceberry	Riceberry	Riceberry	-	Riceberry	Riceberry
clear	low	low	Not clear	low	low	Not clear
texture	very fluid	fluid	cream	very fluid	cream	sticky

Evaluation of stability of the accelerated product by performing 5 rounds of Freeze and thaw cycle.

Table 6 : shows the observation of the separation of layers of skin care products from Riceberry extract.

Cycle	1 (-2)	2 (-1)	3 (middle)	4 (-Ext)	5 (+1)	6 (+2)
1	not separated	not separated	not separated	not separated	not separated	not separated
2	not separated	not separated	not separated	not separated	not separated	not separated
3	not separated	not separated	not separated	not separated	not separated	not separated
4	not separated	not separated	not separated	not separated	not separated	not separated
5	not separated	not separated	not separated	not separated	not separated	not separated

According to the table showing the observation of layer separation of the skincare products from the extract of Riceberry, it was found that all 6 formulations of the extract did not show any layer separation after undergoing 5 rounds of freeze and thaw cycles.

pH value of riceberry sunscreen products

Table 6 : pH value of Riceberry sunscreen products

formula	1st time	2nd time	3rd time
1 (-2)	4.61	4.65	4.64
2(-1)	4.81	4.83	4.79

formula	1st time	2nd time	3rd time
3 (middle)	4.56	4.5	4.55
4 (-Ext)	4.65	4.66	4.60
5 (+1)	4.46	4.44	4.47
6 (+2)	4.67	4.66	4.65

According to the study results, Riceberry extract, and Kojic acid were tested at concentrations ranging from 0.0001 to 1 mg/ml and found to be non-toxic to melanoma cells (B16F10). At a concentration of 1 mg/ml, the cell survival rates were 96.29±2.59% for Riceberry extract and 97.39±1.38% for Kojic acid, respectively. Additionally, Riceberry extract at 1 mg/ml inhibited melanin synthesis in B16F10 cells by 20.60±3.67% but did not affect tyrosinase enzyme activity.

4. Conclusion

In an experiment assessing the toxicity of Riceberry extract and Kojic acid on B16F10 melanoma cells, concentrations from 0.0001 to 1 mg/ml were tested, demonstrating that both substances were non-toxic to the cells. Specifically, at a concentration of 1 mg/ml, the cell survival rate was 96.29±2.59% for Riceberry extract and 97.39±1.38% for Kojic acid. Moreover, Riceberry extract at this concentration decreased melanin synthesis in B16F10 cells by 20.60±3.67%, though it had no impact on tyrosinase enzyme activity.

Additionally, in the evaluation of six formulations of sunscreen, mask, and encapsulated products made from Riceberry extract, formulation 5 proved to be the most stable. It maintained a pH of 4.44±0.03 with no layer separation observed during stability testing.

These findings were part of a preliminary study on the development of sunscreen products using Riceberry extract. Further research and product development are encouraged to explore and validate these initial results.

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