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## ***Exploring Sustainable Skincare Solutions from Sumba Island: The Moisturising and Calming Effects of Aerobadisium pullulans Ferment Isolated from Indonesian Sakura (Cassia javanica)***

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### **1. Introduction**

Indonesia ranks second among the world's megabiodiversity countries based on its vast biodiversity potential. The country harbors 22 types of ecosystems, ranging from terrestrial to marine environments, providing habitats for a wide variety of species. Notably, Indonesia's terrestrial areas are home to approximately 9.70% of the world's flowering plant species. However, only about 3.00% of Indonesia's total biodiversity potential has been utilized to date. Meanwhile, the economic potential of Indonesia's genetic resources is estimated at USD 19.40 billion, accounting for 1.90% of its Gross Domestic Product (GDP) [1].

Sumba, a hidden gem in Indonesia's East Nusa Tenggara, is celebrated for its natural beauty and rich cultural heritage. Known locally as "Humba" or "Hubba," meaning "real," the island offers stunning landscapes that range from rolling hills and pristine beaches to lush savannas and traditional villages. Often regarded as one of Indonesia's most exotic islands, Sumba attracts tourists worldwide who come to experience its unspoiled charm [2-4]

One of Sumba's unique attractions is the Sumba Sakura flower (*Cassia javanica*), locally known as the Konjil flower. Renowned for its delicate pink and white blossoms, the Sumba Sakura blooms during the dry season, typically from September to November, transforming the island's rugged landscapes into a vibrant display of color. These wildflowers adorn the roads and hills of East Sumba, especially in areas like Waingapu and Tanggedu. Their striking beauty has made them a popular destination for tourists visiting the island during the flowering season to capture stunning photographs among these vibrant blossoms, complementing Sumba's exotic scenery [5] [6].

The threat of global species extinction has garnered significant attention from various stakeholders. At the global level, at least one million species are currently facing extinction, partly due to excessive exploitation. To ensure the preservation of biodiversity for future generations, sustainable management practices are crucial [1]

The Sumba Sakura was chosen as the focus of this study because of its aesthetic appeal as a symbol of Sumba Island—a place of raw elegance and untamed allure. Both the island

and its sakura embody a harmonious blend of resilience and delicacy, thriving in the challenging conditions of Sumba's dry, rocky soil while displaying exceptional visual beauty.

To preserve the population and still get the benefit of Sumba Sakura, *Aureobasidium pullulans*, a resilient yeast-like fungus, was isolated and fermented to produce a bioactive extract. This ferment is rich in antioxidants, polysaccharides, and other metabolites that offer significant skin benefits, including hydration, elasticity, and protection against environmental stressors. The combination of the Sumba Sakura flower's natural beauty and the innovative potential of *A. pullulans* ferment highlights the underexplored value of Sumba's biodiversity in advancing modern skincare.

To preserve the population and still get the benefit of Sumba Sakura, *Aureobasidium pullulans*, a resilient yeast-like fungus, was isolated and fermented to produce a bioactive extract. This ferment is rich in antioxidants, polysaccharides, and other metabolites that offer significant skin benefits, including hydration, elasticity, and protection against environmental stressors. Specifically, *A. pullulans* has been shown to produce pullulan, a polysaccharide with moisturizing properties, and metabolites with antioxidant effects that protect skin cells from oxidative damage and UV-induced stress [8][9]. Furthermore, *A. pullulans* lysate has demonstrated protective effects against UV-damaged human skin fibroblasts and HaCaT cells, supporting its role in enhancing skin barrier function and reducing signs of aging [9]. The combination of the Sumba Sakura flower's natural beauty and the innovative potential of *A. pullulans* ferment highlights the underexplored value of Sumba's biodiversity in advancing modern skincare, suggesting that the indigenous flora of the region can contribute to both sustainable practices and advanced cosmetic formulations. This underscores the growing potential of *A. pullulans* as an effective bioactive ingredient in skincare, much like other bioactive plant-derived components explored in recent studies [7].

By combining Sumba's natural beauty with advanced scientific exploration, this research showcases the moisturizing and calming effects of *Aureobasidium pullulans* ferment derived from the enchanting Sumba Sakura, offering a fresh perspective on the island's natural resources.

## 2. Materials and Methods

### 2.1 Materials

The materials used in this study included *Aureobasidium pullulans* isolated from Sumba Sakura, cultured in an optimized medium to enhance exopolysaccharide (EPS) production. The cream based formulation consisted of water, glycerin, pentylene glycol, Methylpropanediol, Hydroxyethyl Acrylate/Sodium Acryloyldimethyl Taurate Copolymer, glyceryl stearate, cetearyl olivate, caprylic/capric triglyceride, ethylhexyl palmitate, and shea butter. For in vitro assays, L-NMMA was used for nitric oxide inhibition, and 1.2 mM calcium chloride ( $\text{CaCl}_2$ ) was used to measure filaggrin mRNA expression. In vivo evaluations involved 2% Sumba sakura extract for skin irritation tests, an SLS-induced irritation model, and a Corneometer to assess hydration levels.

### 2.2 Method

#### 2.2.1 Isolation of *Aureobasidium Pullulans* from Sumba Sakura

*Aureobasidium pullulans* was isolated from Sumba sakura using high-throughput screening (HTS) technology, which enabled rapid separation of the target microorganism

within a short time. The isolated strain was cultivated under controlled fermentation conditions to enhance its suitability for cosmetic applications. The culture medium was optimized to suppress melanin production, resulting in the growth of pink-colored *Aureobasidium* strains. To maximize the production of exopolysaccharides (EPS), including pullulan, high-density culture (HDC) technology was applied by controlling the medium composition to increase EPS yield. The culture supernatant containing EPS was then purified using Bio-Puri technology through ultrafiltration, effectively removing medium components and microbial cells and yielding purified EPS with a uniform molecular profile. Finally, the purified EPS was concentrated via precipitation to obtain a concentrated pullulan product suitable for further application.

## 2.2.2 Preparation and Formulation of Sumba sakura Based-Cream for In Vivo Evaluation

The Sumba sakura sample used in this study was prepared by incorporating 1% *Aureobasidium pullulans* into a solution containing 86.95% water, 10% propanediol, 2% 1,2-hexanediol, and 0.05% ethylhexylglycerin. The mixture was thoroughly homogenized and subsequently utilized in the clinical study. The cream formulation was prepared according to the following composition:

Part	Material Name	%
	Water	69.1
	Glycerin	4
	Pentylene Glycol	3
	Methylpropanediol	1
A	<i>Aureobasidium Pullulans</i> Ferment (and) Propanediol (and) 1,2-Hexanediol (and) Ethylhexylglycerin	2
	Hydroxyethyl Acrylate/Sodium	
B	Acryloyldimethyl Taurate Copolymer	0.4
	Glyceryl Stearate	1.5
	Cetearyl Olivatate (and) Sorbitan Olivatate	2
	Caprylic/Capric Triglyceride	1
C	Ethylhexyl Palmitate	11
	<i>Butyrospermum Parkii</i> (Shea Butter)	5

**Tabel 1.** Formulation of Sumba sakura Based-Cream

## 2.2.3 Efficacy Evaluation

### 2.2.3.1 In Vitro Evaluation

#### a. Anti-Inflammation

The anti-inflammatory activity was assessed through the inhibition of nitric oxide (NO) production assay. The experimental setup included three groups: (1) a positive control treated with L-NMMA, a known NO synthase inhibitor; (2) a negative control consisting of cells stimulated with lipopolysaccharide (LPS) without any treatment; and (3) an untreated control consisting of cells without LPS stimulation and without any treatment.

## b. Filaggrin mRNA Expression Assay

The effect on skin barrier function was evaluated by measuring filaggrin mRNA expression levels. The experiment included three groups: (1) a positive control treated with 1.2 mM calcium chloride ( $\text{CaCl}_2$ ), known to upregulate filaggrin expression; (2) a negative control consisting of untreated cells; and (3) the test samples.

### 2.2.3.2 In Vivo Evaluation

#### a. Primary Skin Irritation Test

The study was performed using a cream base containing 2% Sumba sakura extract, a solution of 2% Sumba sakura extract in distilled water, and an untreated control. A total of 8 participants were involved in the study. Skin irritation was evaluated at 30 minutes, 24 hours, and 48 hours post-application by assessing visual signs of erythema and edema at each time point.

#### b. Induced Skin Irritation Test

A skin irritation test was conducted using an SLS-induced irritation model. A cream base containing 2% *Sumba sakura* extract and a placebo cream base (without *Sumba sakura*) as the untreated control were applied to the skin of eight healthy participants. Skin reactions were assessed at baseline (0 hours), and after 24, 48, and 72 hours of application.

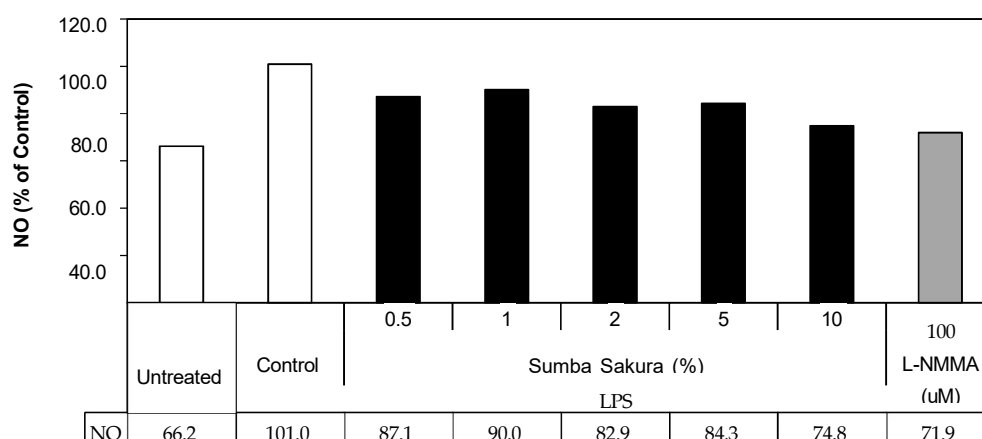
#### c. Long-term Moisturizing Test

A long-term moisturizing efficacy test was performed with four participants using a Cream Base containing either Sumba sakura or distilled water, both at a concentration of 2%. The samples were applied twice daily, in the morning and evening, on the inner part of the forearm, with a sample volume of 25  $\mu\text{L}$ . Hydration was measured weekly using a Corneometer.

## 3. Results

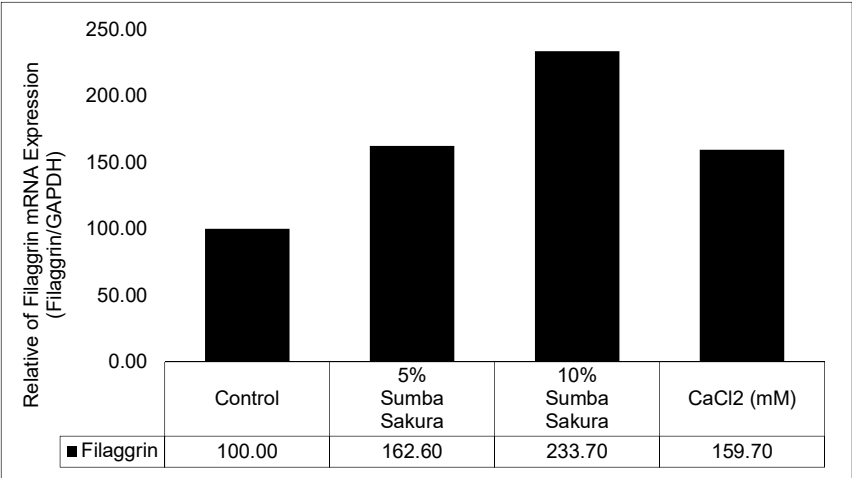
### 3.1 In vitro Evaluation

#### 3.1.1 Anti-Inflammation



**Figure 1.** Nitric Oxide Inhibition by Sumba sakura (0.5–10%) Compared to Positive Control, Negative Control and Untreated Control

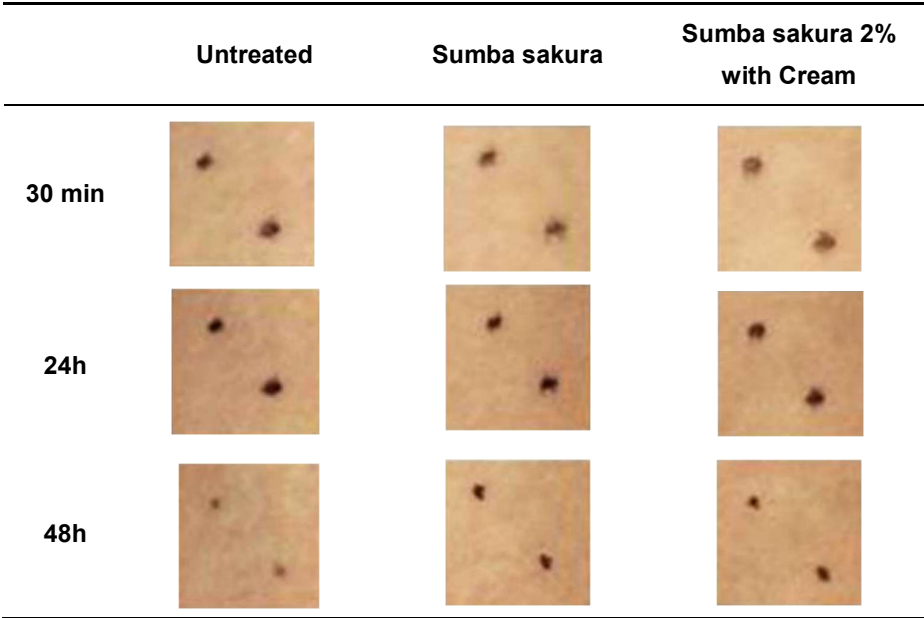
3.1.2 Filaggrin mRNA Expression Assay



**Figure 2.** Relative Filaggrin mRNA expression of Sumba Sakura at 5% and 10% compared to Negative and Positive Control

3.2 In Vivo Evaluation

3.2.1 Primary Skin Irritation Test

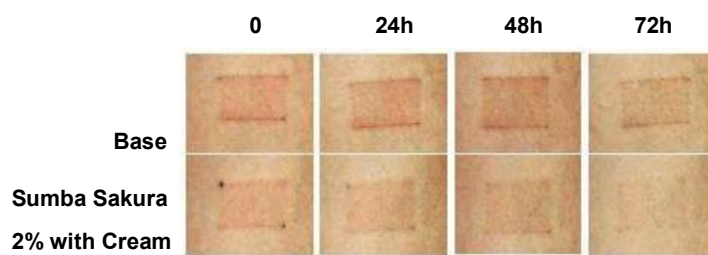


**Figure 3.** No skin erythema was observed at any assessed time point in the untreated group, the Sumba sakura group, or the group treated with 2% Sumba sakura cream

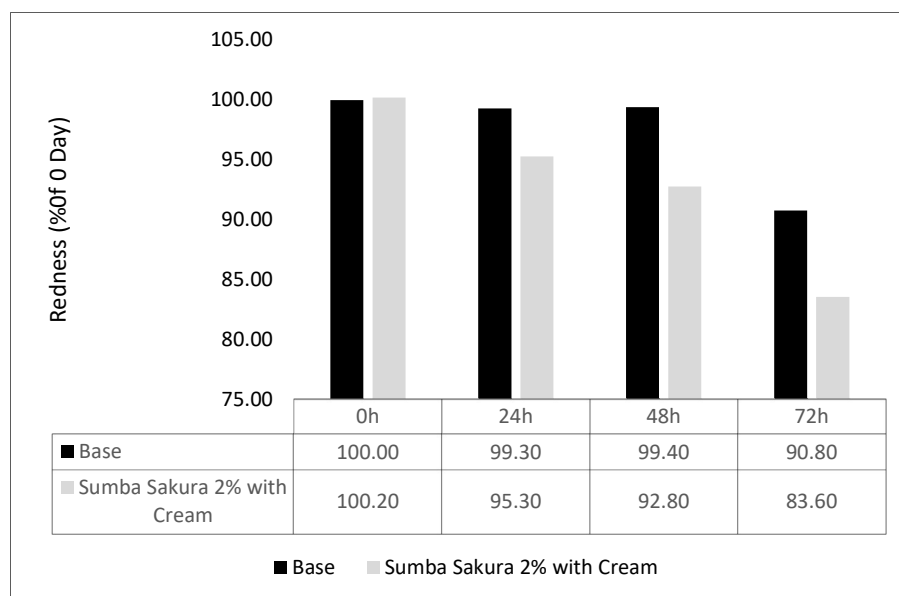
	Untreated			Sumba Sakura			Sumba Sakura 2% with Cream		
	30 min	24h	48h	30 min	24h	48h	30 min	24h	48h
Subject 1	-	-	-	-	-	-	-	-	-
Subject 2	-	-	-	-	-	-	-	-	-
Subject 3	-	-	-	-	-	-	-	-	-
Subject 4	-	-	-	-	-	-	-	-	-
±	0	0	0	0	0	0	0	0	0
+	0	0	0	0	0	0	0	0	0
++	0	0	0	0	0	0	0	0	0
Mean Score	0.00			0.00			0.00		
Assessment	No Stimulus			No Stimulus			No Stimulus		

**Table 2.** Primary Skin Irritation Result of the untreated, Sumba Sakura and Sumba Sakura 2% with Cream

### 3.2.2 Induced Skin Irritation Test

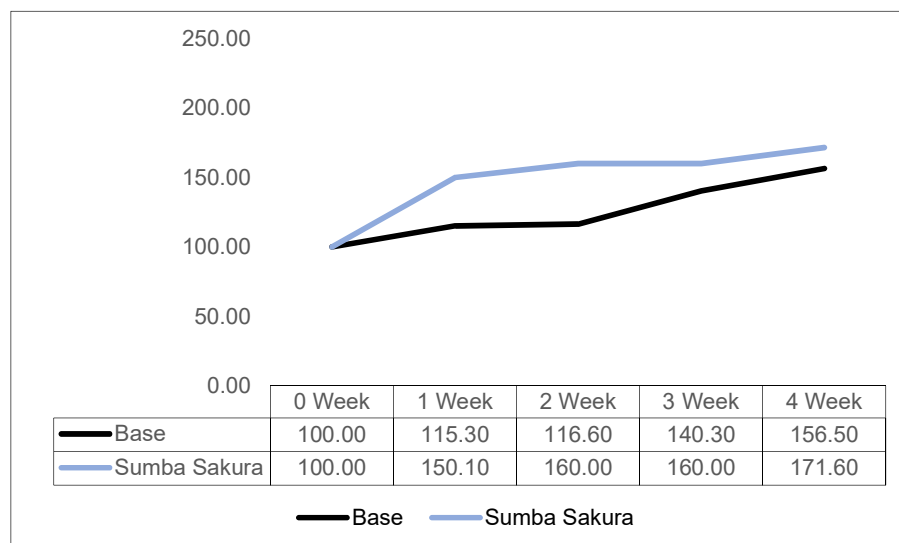


**Figure 4.** No skin erythema was observed at any assessed time point in the Base or the group treated with 2% Sumba sakura cream



**Figure 5.** Induced Skin Irritation Result of the base and Sumba Sakura 2% with Crea

### 3.2.3 Long-term Moisturizing Test



**Figure 6.** Moisture Content (%) Result of Sumba Sakura compare with base

## 4. Discussion

The results of this study demonstrate the multifunctional skin benefits of *Sumba Sakura* extract through a series of in vitro and in vivo evaluations. The anti-inflammatory potential of *Sumba Sakura* was evidenced by its ability to inhibit nitric oxide (NO) production in LPS-induced macrophages. Across concentrations ranging from 0.5% to 10%, the inhibition rates varied from 12.9% to 26.2%, with the highest activity observed at 10% (26.2%), approaching the effect of the positive control (29.1%). This suggests that *Sumba Sakura* possesses appreciable anti-inflammatory properties, although a concentration-dependent trend was not clearly observed.

Furthermore, the skin barrier enhancement activity was demonstrated through the upregulation of filaggrin mRNA expression. Application of *Sumba Sakura* at 5% and 10% concentrations resulted in a 162.6% and 233.7% increase, respectively, surpassing the  $\text{CaCl}_2$  (1.2 mM) positive control, which showed a 159.7% increase. This indicates a strong potential of *Sumba Sakura* in promoting skin barrier function by enhancing filaggrin expression, a key protein in epidermal differentiation and barrier integrity.

In vivo skin tolerance testing confirmed the safety of *Sumba Sakura*. Neither the raw material nor the cream containing 2% *Sumba Sakura* elicited any primary irritation responses in the single patch test, comparable to untreated skin. These findings support the claim that *Sumba Sakura* is a non-irritant and suitable for topical application.

The calming effect of *Sumba Sakura* was verified in an SLS-induced irritation model. A cream containing 2% *Sumba Sakura* reduced skin redness by 16.4% after 3 days of application, compared to a 9.2% reduction with the base formulation. This significant improvement highlights the soothing efficacy of *Sumba Sakura* as a calming agent.

Lastly, the long-term moisturizing effect was demonstrated by a 71.6% increase in skin hydration after 4 weeks of application with the *Sumba Sakura* 2% cream, compared to a 56.5% improvement observed with the control product. This indicates the promising hydrating property of *Sumba Sakura*, further supporting its potential as a multifunctional cosmetic ingredient.

Overall, these findings suggest that *Sumba Sakura* is a safe and effective natural ingredient with anti-inflammatory, barrier-enhancing, soothing, and moisturizing properties, making it a promising candidate for cosmetic formulations targeting sensitive and dry skin conditions.

## 5. Conclusion

This study demonstrated that *Aerobasidium Pullulans* Ferment isolated from Sumba Sakura (*Cassia Javanica*) offers multifunctional skin benefits, including anti-inflammatory, barrier-enhancing, soothing, and moisturizing effects, with proven safety for topical use. These results highlight its potential as a sustainable and effective natural ingredient for cosmetic formulations, while promoting the value of Indonesia's native biodiversity.

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