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"Research of Mung bean sphingomyelin extract promoting skin barrier repair"

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1. Introduction

Sphingolipids constitute approximately 40% of the lipid composition in the plasma membrane [1,2] and are also prevalent within the endomembrane system [3]. In the medical field, sphingolipids play a crucial role in neurodegenerative diseases linked to insulin resistance in the brain. Recent evidence has substantiated that notable alterations in sphingolipids and their metabolic pathways correlate significantly with various neurological disorders including Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), Gaucher disease, and Farber disease [4]. On the other hand, Sphingolipids have been found to accumulate in long-living cohort of human population and were declared as markers and biological modulators of healthy aging in humans by providing a better antioxidant capacity and participating in membrane remodeling process [5,6].

By contrast with animals, sphingomyelin, globosides, sulfatides or gangliosides are absent in plants. The complex structural diversity of sphingolipids arises from the possible occurrence of three building blocks connected to the sphingoid backbone: a polar head, a fatty acyl chain (FA) linked by an amide bond (to form a ceramide) to a long-chain amino-alcohol called long-chain base (LCB). However, plants and fungi do not possess these sphingolipids. Instead, they contain complex glycosphingolipids known as glycosyl inositol phosphoryl ceramides (GIPC). The GIPCs, displaying a similar structural with animal gangliosides, consists of a ceramide moiety linked to an in-ositol–glucuronic acid unit via a phosphodiester bond. Similarity in structure between animals and plants indicated the similarity function.

Recent investigations have revealed that plant sphingolipids appear as a promis-ing class of components susceptible to prevent the onset of the metabolic syndrome (MetS). In 2020, Hermier et al. [7] demonstrated that camelina sphingomyelin could markedly alleviate obesity and insulin resistance metabolism parameters， as well as indicators of inflammation and dysfunctions related to colonic barrier integrity. At the same year, Yamashita et al.[8] reported that each mushroom polar lipid fraction suppressed lipopolysaccharide (LPS)-induced decreases in the viability of intestinal cells, and the effects of sphingolipid fractions were significantly stronger than those of fraction that did not contain sphingolipids[9]. Additionally, Mushroom sphingolipids could suppress intestinal apoptosis induced by inflammatory stress, and highly polar sphin-golipids may exert stronger inhibitory capabilities[10]. Although plant

sphingomyelin had been proved to play an important role in the metabolic syndrome (MetS), there is few knowledge about its function in skin care.

Herein, we extract sphingomyelin from germinating mung bean using Sephadex column chromatography followed by TLC. Using the isolated sphingomyelins (Mb-SE), we then examined their skin barrier repair ability in skin cells. The efficacy was as-sessed using human epidermal Hacat cell lines, establishing a foundation for further investigation into the mechanisms underlying the effects of plant-derived sphingomyelin in skin health. This research offers novel insights into the antioxidant properties, barrier repair capabilities, and soothing effects of plant sphin-golipids on human skin and contributes to advancements in skincare within both medical and cosmetic fields.

2. Materials and Methods

2.1 Extraction of Mung Bean Sphingolipids (Mb-SE)

Following the sphingolipid extraction method described by Jonathan E. Markham[11] in his 2006 paper, fresh mung bean sprouts were crushed and pressed to remove the sap. An extraction solution (isopropanol: n-hexane: water = 55:20:25) was added at a solid-liquid ratio of 1:3, and extraction was performed at 60°C for 1-3 hours, repeated twice. The extract was filtered using a chromatography column (0.2 μm PP filter) and collected. After concentrating at 55-60°C, the extract was freeze-dried to obtain extraction dry powder samples, which are the mung bean sphingolipid extract samples (Mb-SE).

2.2 HPTLC Method for Sphingolipid Detection

Based on the HPTLC detection method for sphingolipids reported by Johannes Müthing [12] in 2009, this study used high-performance thin-layer chromatography silica gel plates HSGF254 type (5.0*10.0 cm). The developing buffer was $CHCl_3$/CH_3OH/H_2O (65:25:4, by vol.). After drying the developed layer, it was sprayed with alkaline potassium permanganate and heated to dry. Lipid compounds appeared as yellow spots against a light red background.

2.3 MTT Cytotoxicity Test

This study used the MTT method, which involves the reaction of MTT with succinate dehydrogenase in active cell mitochondria to form blue-purple crystalline formazan deposited in cells, to assess the cytotoxicity of exogenous substances [13]. Human skin fibroblast cell suspension density was adjusted to 0.8×10^5 cells/mL and seeded into a 96-well plate, cultured at 37°C in a 5% CO_2 incubator for 24 hours. Samples without mung bean sphingolipid extract served as the negative control group, SEPPIC wheat sphingolipid extract as the positive control group, and different concentrations of mung bean sphingolipid extract (F1-F3) as the experimental group (4 samples). After 24 hours of incubation at 37°C in a 5% CO_2 incubator, cell morphology was observed and photographed under a microscope. In a light-protected environment, 10 μL of MTT solution (5 mg/mL) was added to each well, reacted for 4 hours, and the supernatant was discarded. Then, 150 μL of DMSO was added and shaken to dissolve for 10 minutes. Absorbance values at 570 nm were measured using a microplate

reader, with 630 nm as the reference wavelength. The cell viability percentage was calculated using the formula: Cell viability percentage (%) = (average OD value of sample wells / average OD value of negative control group)×100%. Data were statistically analyzed and graphed using GraphPad Prism 8.0 software.

2.4 Barrier Repair Test FLG/LOR

An improved skin barrier repair detection method was used for testing [14]. HaCaT cells were seeded and suspension density adjusted to 1.5×10^4 cells/well, cultured at 37°C in a 5% CO_2 incubator for 24 hours. Samples without mung bean sphingolipid extract served as the negative control group, 50 mM EGCG as the positive control group, and different concentrations of mung bean sphingolipid extract (F1-F3) as the experimental group (4 samples). After 24 hours of incubation at 37°C in a 5% CO_2 incubator, cells were collected. Treated HaCaT cells were fixed with 4% paraformaldehyde and blocked, then incubated with FLG/LOR antibodies, followed by secondary incubation with fluorescent secondary antibodies. Observations and photographs were taken using a fluorescence microscope. Average fluorescence intensity analysis was performed using ImageJ, and data were statistically analyzed and graphed using GraphPad Prism 8.0 software.

3. Results

3.1. Mung Bean Sphingolipids（Mb-SE）Exhibits No Significant Cytotoxicity in HaCaT Cell Model

Cytotoxicity refers to the simple cell-killing events caused by cells or chemicals, independent of apoptosis or necrosis mechanisms. Cytotoxicity testing is primarily conducted by detecting changes in cell membrane permeability, with methods including MTT, XTT, and LDH assays. In this project, the MTT method was used for cytotoxicity testing. This method is based on the principle that the highly active succinate dehydrogenase in normal cell mitochondria can reduce tetrazolium salts like MTT to form purple crystalline substances, which can be measured by a microplate reader to reflect cell proliferation activity, thereby indicating the cytotoxicity of Mung bean sphingolipids（Mb-SE）.

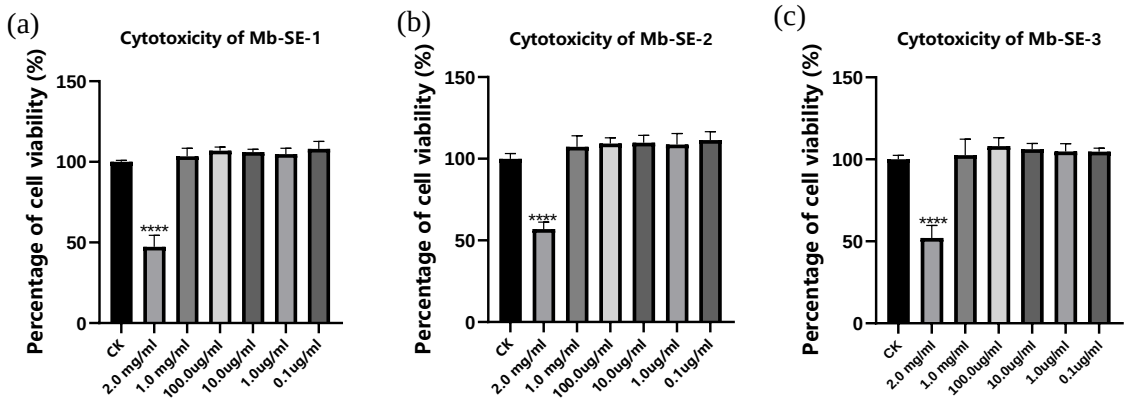

Figure 1 Cytotoxicity detection of Mung Bean Sphingolipids（Mb-SE）. Data are the means ± SD of four biological repeats. Asterisks indicate the statistically significant difference levels compared with the wild-type plants (Student's t-test: *, $P < 0.05$; **, $P < 0.01$).

In this project, wheat sphingolipid material was selected as the reference group. Five groups of samples, including the solvent group (propylene glycol: water, 9:1), three batches of Mb-SE samples, and the reference group, were tested for cytotoxicity at concentrations ranging from 0.1 ug/ul to 2.0 mg/ml. The results showed that the solvent at a concentration of 2.0 mg/ml exhibited strong cytotoxicity, but the reference group samples and the three Mb-SE did not significantly inhibit cell proliferation activity (FIG). Therefore, the Mung Bean Sphingolipids（Mb-SE） did not exhibit obvious cytotoxicity in Hacat cells and was considered safe at the cellular level.

3.2 Mung Bean Sphingolipids（Mb-SE） Significantly Suppresses ROS Generation and Enhances Total Antioxidant Capacity in HaCaT Cells

The human body continuously generates free radicals due to external interactions, including respiration (oxidative reactions), external pollution, and radiation exposure. Increasing research indicates that antioxidation is a crucial step in preventing aging, as free radicals or oxidants can decompose cells and tissues, affecting metabolic functions and causing various health issues [15]. Eliminating excessive oxidative free radicals can prevent many free radical-induced and aging-related diseases. Therefore, antioxidation is a key research

indicator for anti-aging and is a primary research direction for health and cosmetic companies, as well as a major functional demand in the market [16].

This project evaluated the comprehensive antioxidant capacity of Mb-SE from two perspectives: ROS production and antioxidant capacity (T-AOC). First, using human immortalized keratinocytes (Hacat cells) as the research object, the antioxidant capacity of different Mb-SE against H_2O_2 oxidative damage was assessed using ROS fluorescence probe and ROS fluorescence absorbance methods. The results showed that, compared to the control group (VC group), the fluorescence intensity in Hacat cells significantly decreased for the three Mb-SE (-1, -2, and -3) and wheat glycolipid material after H_2O_2 oxidative damage (Figure 3A). Statistical data indicated a significant reduction in ROS content (Figure 3B), demonstrating that Mb-SE can significantly inhibit ROS formation.

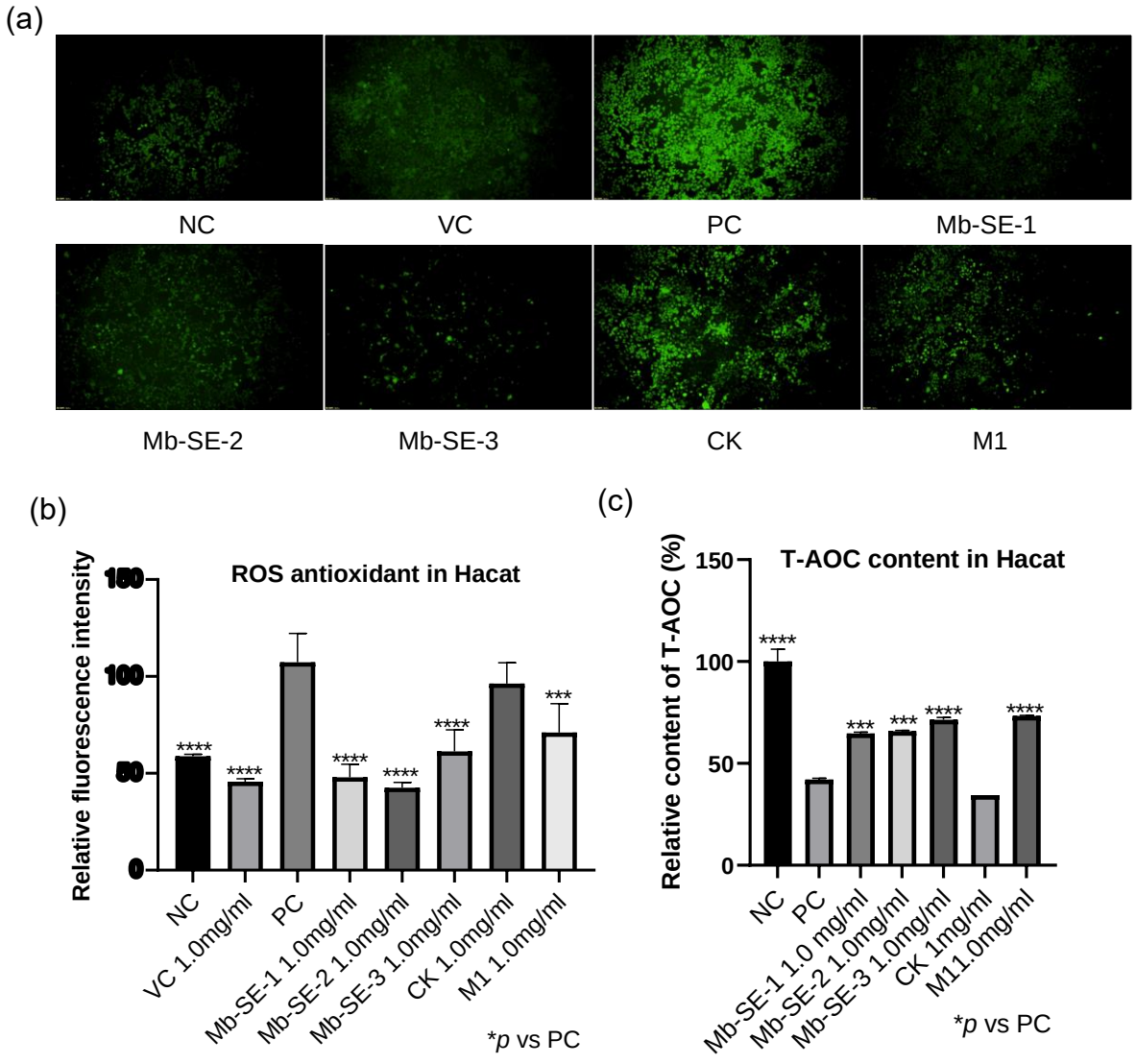

Figure 2 Antioxidant detection of GIPC extracts from mung bean sprout. Data are the means ± SD of four biological repeats. Asterisks indicate the statistically significant difference levels compared with the wild-type plants (Student's t-test: ***, P < 0.001; ****, P < 0.0001).

Secondly, the total antioxidant capacity (T-AOC) refers to the overall antioxidant level composed of various antioxidants and antioxidant enzymes, such as antioxidant enzymes, vitamin C, vitamin E, and carotenoids, which protect cells and the body from oxidative stress damage caused by reactive oxygen species. Therefore, total antioxidant capacity can be used to evaluate the antioxidant capacity of bioactive substances. This project also analyzed the total antioxidant capacity (T-AOC) of the three Mb-SE (-1, -2, and -3). The results showed that, compared to the control group, the relative T-AOC content of the three Mb-SE (-1, -2, and -3) and wheat glycolipid material significantly increased (Figure 2c), indicating that the Mb-SE have good antioxidant capacity.

3.3 Mung Bean Sphingolipids (Mb-SE) Unveils Skin Barrier Repair Potential via Dual Modulation of FLG and LOR Expression

FLG and LOR are important indicators affecting skin barrier function [17]. FLG can bind to intermediate filaments of cell keratin, providing a framework for epidermal cells, while LOR is mainly located between the granular and stratum corneum layers, constituting 70% of the total epidermal keratin. Together, they form the local microen-vironment of epidermal cells, influencing their growth and metabolism. When FLG and LOR expression decreases in epidermal tissue, the water content between skin matrices reduces, making the epidermis prone to shedding, increasing transepidermal water loss, exacerbating skin dryness, and further affecting normal epidermal cell proliferation and metabolism. In this project, a 500 μg/ml EGCG concentration was used for modeling as a positive control group. The three Mb-SE (-1, -2, and -3) samples were used for modeling, and LOR expression was detected after 24 hours of recovery.

The FLG test results showed that at a concentration of 0.1mg/ml, the three Mb-SE (-1, -2, and -3) samples significantly increased the expression of filaggrin (FLG) in Hacat cells; at a concentration of 1.0mg/ml, there was a highly significant upward trend (Figure 3a and 3c); two batches of 0.5mg/ml Mb-SE (-1 and -2) samples also significantly increased the expression level of loricrin (LOR) in Hacat cells (Figure 3b and 3d). In summary, Mb-SE have a significant skin barrier protection potential.

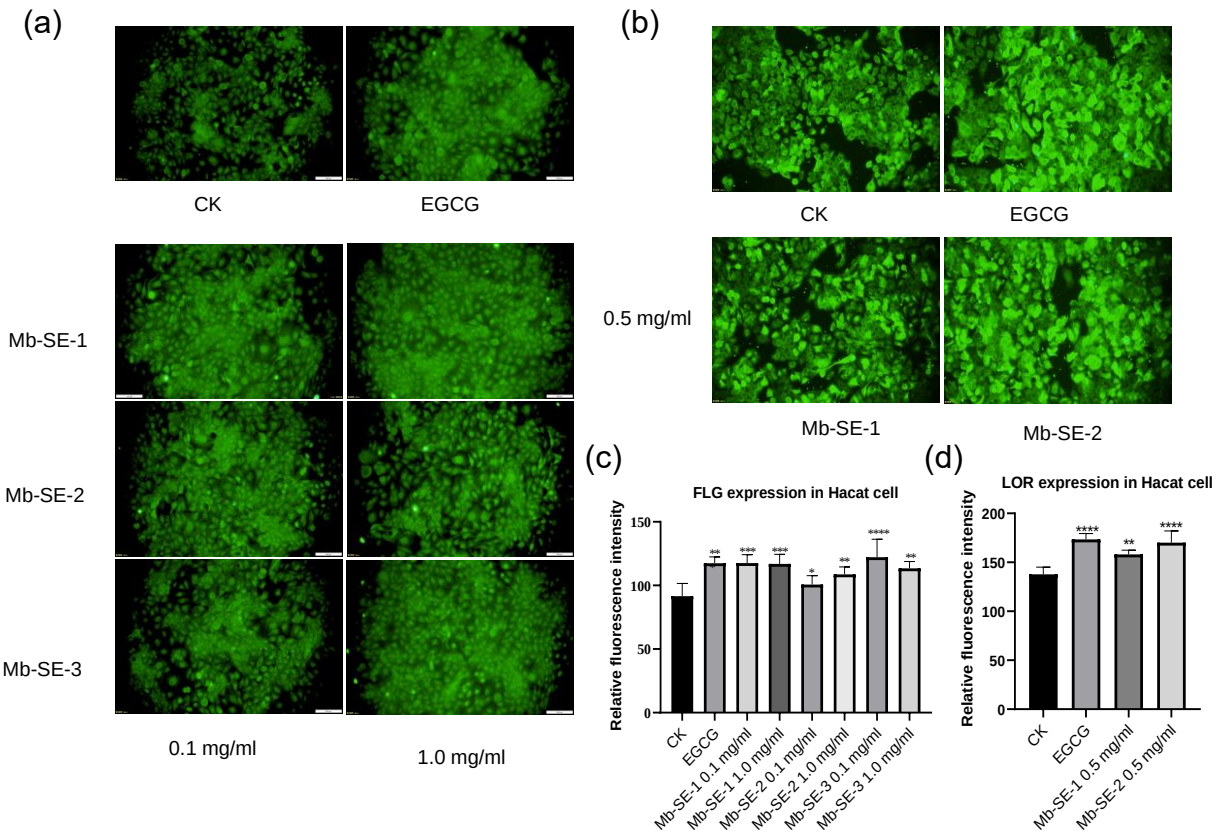

Figure 3 Expression analysis of FLG/LOR in Hacat Cells. Data are the means ± SD of four biological repeats. Asterisks indicate the statistically significant difference levels compared with the wild-type plants (Student's t-test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$).

4. Discussion

4.1 Synergistic Safety and Efficacy of Mung Bean Sphingolipids (Mb-SE) Across Models

The Mb-SE demonstrated a remarkable balance between safety and bioactivity. Cytotoxicity assays (MTT method) confirmed their cellular safety up to 2.0mg/mL in Hacat cells, with no significant inhibition of proliferation, while the solvent control (propylene glycol:water, 9:1) exhibited acute cytotoxicity at this concentration. This safety profile aligns with their potent antioxidant and barrier-repairing effects. For instance, the extracts significantly reduced ROS levels (via ROS fluorescence assays) and enhanced total antioxidant capacity (T-AOC) in Hacat cells under oxidative stress, comparable to VC controls. Furthermore, low concentrations (0.01–0.1%) of Mb-SE upregulated FLG and LOR expression-critical structural proteins for epidermal integrity—suggesting a dose-dependent mechanism for barrier repair. These findings collectively highlight Mb-SE as multifunctional candidates for cosmetic formulations, where safety and efficacy are non-negotiable.

4.2 Implications for Bioactive Optimization and Mechanistic Exploration

While the study establishes GIPC extracts as promising bioactive agents, key questions remain. For example, the specific components within the extracts (e.g., sphingolipid subclasses or glycosylated derivatives) responsible for ROS inhibition, T-AOC enhancement, and FLG/LOR upregulation are yet to be identified. Additionally, the interplay between these mechanisms—such as whether antioxidant activity directly influences barrier protein expression via redox-sensitive signaling pathways (e.g., Nrf2/ARE)—warrants investigation. Future studies should employ metabolomic profiling and pathway-specific inhibitors to dissect these relationships. Furthermore, formulation studies are needed to assess stability and bioavailability in topical applications, as the current in vitro and embryonic models do not fully replicate human skin physiology.

5. Conclusion

The Mb-SE exhibit a unique triad of safety, antioxidant potency, and barrier repair efficacy, validated across cellular and vertebrate models. Their multimodal action positions them as innovative candidates for anti-aging, sensitive skin care, and barrier-enhancing formulations. Addressing mechanistic and translational gaps will solidify their commercial and therapeutic potential.

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