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RESEARCH ARTICLE
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ECTOINE FROM *HALOMONAS DAQINGENSIS* DSH-3: CYTOTOXICITY AND PROTECTIVE POTENTIAL

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Abstract:
Background: Ectoine is a protective solute synthesized by extremophilic bacteria and is gaining attention for its potential use in dermatology and cosmetics due to its ability to safeguard cells from stress.
Objectives: This study investigated the cytotoxic and protective effects of ectoine extracted from *Halomonas daqingensis* strain DSH-3 on human keratinocytes (HaCaT) and melanoma cells (B16-F10).
Methods: Ectoine was extracted and evaluated in vitro. Cytotoxicity was determined using the MTT assay, while antioxidant activity was assessed by measuring GSH-Px and SOD levels under LPS-induced oxidative stress. Skin-lightening potential was examined through mushroom tyrosinase inhibition and UV-induced melanin synthesis assays.
Results: The MTT assay demonstrated a dose-dependent cytotoxicity profile, with cell viability remaining above 99% at the lowest tested concentration (7.8 µg/mL) but declining at higher doses. Antioxidant assays showed a significant increase in GSH-Px and SOD activity, confirming the cytoprotective properties of ectoine. Although the compound displayed limited mushroom tyrosinase inhibition (IC_{50} > 1000 µg/mL), it effectively reduced UV-induced melanin synthesis in keratinocytes by 51.02% at 500 µg/mL.
Conclusion: Ectoine from *H. daqingensis* DSH-3 exhibits strong antioxidant and cytoprotective activities with promising applications in managing oxidative stress and hyperpigmentation. However, its role as a direct tyrosinase inhibitor appears limited.

Keywords: ectoine, *Halomonas daqingensis*, cytotoxicity, HaCaT, B16-F10, antioxidant, melanin inhibition, skin lightening, MTT assay, LPS stress

1. Introduction

In order to protect themselves against environmental stressors such high salinity, harsh temperatures, UV light and oxidative stress, halophilic and extremophilic bacteria produce ectoine, a naturally occurring suitable solute. Initially discovered in *Ectothiorhodospira halochloris*, ectoine has gained considerable interest for its unique protective properties and its biocompatibility with human tissues (Galinski et al., 1985; Graf et al., 2008). This small cyclic amino acid (1,4,5,6-tetrahydro-2-methyl-4-pyrimidine carboxylic acid) stabilizes proteins, membranes and nucleic acids without interfering with the biochemical processes of the cell (Arakawa & Timasheff, 1985; Lentzen & Schwarz, 2006).

In recent years, ectoine has emerged as a valuable bioactive molecule in dermatology and cosmetology due to its cytoprotective, antioxidant and anti-inflammatory activities. It has been shown to reduce UV-induced damage, inhibit reactive oxygen species (ROS), and promote skin hydration and regeneration (Buommino et al., 2021). Additionally, ectoine modulates the skin's stress response pathways and has been reported to inhibit melanin synthesis in melanocytes, offering potential application in skin whitening and anti-pigmentation products (Lee et al., 2019).

Halophilic bacteria, particularly members of the genus *Halomonas*, are recognized for their efficient ectoine production under saline stress conditions. Among them, *Halomonas daqingensis* DSH-3 has demonstrated a promising capability to synthesize ectoine when cultivated under optimized NaCl concentrations, suggesting its potential as a sustainable microbial factory for biotechnological applications.

Despite growing interest, limited studies have evaluated the in vitro effects of microbially produced ectoine on skin-relevant cellular models such as human keratinocytes (HaCaT cells) and melanoma cells (B16-F10). Therefore, this study aims to evaluate the cytotoxicity, antioxidant activity and melanin inhibition potential of ectoine extracted from *Halomonas daqingensis* DSH-3. The findings from this research are expected to provide scientific evidence supporting the safe and effective use of bacterial ectoine in cosmetic and therapeutic skincare formulations.

2. Materials and Methods

2.1. Test Product and Cell Lines

Ectoine used in this study was isolated and purified from the halophilic bacterium *Halomonas daqingensis* strain DSH-3, previously cultured under optimized saline conditions to enhance ectoine production. The bacterial biomass was harvested, lysed, and subjected to high-performance liquid chromatography (HPLC) purification to isolate ectoine with >98% purity. The identity and concentration of ectoine were confirmed using ^1H NMR and mass spectrometry techniques as described by Pastor et al. (2010).

For in vitro experiments, two cell lines were employed:

- Human keratinocyte cell line (HaCaT) – widely used as a model for skin barrier function and wound healing.
- Mouse melanoma cell line (B16-F10) – commonly utilized in pigmentation and melanogenesis research.

The National Centre for Cell Science (NCCS), located in Pune, India, provided both cell lines. The cells were kept at 37°C in a humidified environment with 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin.

2.2. Cytotoxicity Assay (MTT Assay)

In accordance with Mosmann's (1983) methodology, the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] test was used to assess the cytotoxicity of ectoine on HaCaT and B16-F10 cells. In short, 96-well plates were seeded with 1×10^4 cells per well, and the cells were then incubated throughout the entire night. After that, they received a 24-hour and 48hrs, the course of ectoine at successive doses ranging from 7.8 to 1000 µg/mL. After incubation, each well received 10

µL of MTT solution (5 mg/mL in PBS) and was left to incubate for four hours. A microplate reader was used to assess absorbance at 570 nm after formazan crystals produced by metabolically active cells were dissolved in DMSO. The percentage of cells that survived treatment was compared to the untreated control.

2.3. Morphological Assessment of HaCaT Cells

The HaCaT cells were seeded in 6-well plates and exposed to ectoine at progressively higher concentrations (125–500 µg/mL) in order to assess the impact of ectoine on cell shape. After a day, morphological changes were seen using an Olympus CKX53 inverted phase-contrast microscope in Tokyo, Japan. Pictures were taken with magnifications of 10× and 20×. To determine cytotoxic or protective effects, morphological markers such membrane blebbing, cell shrinkage and detachment were evaluated.

2.4. Assay for Tyrosinase Inhibition

The mushroom tyrosinase assay, as outlined by Masuda et al. (2005), was used to evaluate the inhibitory effect of ectoine on tyrosinase activity. In short, a 96-well plate was filled with 120 µL of phosphate buffer (50 mM, pH 6.8), 20 µL of ectoine solution (final concentrations 100–500 µg/mL), 20 µL of mushroom tyrosinase (200 units/mL) and 20 µL of L-DOPA (2 mM). Kojic acid was used as the positive control at 100 µg/mL. The absorbance at 475 nm was measured after the reaction mixture was incubated for 30 minutes at 37 °C. Tyrosinase activity inhibition as a percentage of control was computed.

2.5. Antioxidant Activity Under LPS-Induced Oxidative Stress

To evaluate intracellular antioxidant activity, HaCaT cells were stimulated with lipopolysaccharide (LPS, 1 µg/mL) to induce oxidative stress for 3 hours, followed by treatment with ectoine (250 and 500 µg/mL) or the antioxidant standard Quercetin (250 µg/mL) for 24 hours. Post-treatment, cellular lysates were collected, and the activities of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) were quantified using commercially available assay kits (Sigma-Aldrich, USA), observing the guidelines provided by the manufacturer. Enzymatic activity was reported in U/mg protein and adjusted to the total protein content.

2.6. Melanin Inhibition Assay (UV-Induced Melanogenesis)

To determine the anti-pigmentation potential of ectoine, B16-F10 melanoma cells were seeded in 6-well plates and pre-treated with ectoine (125–500 µg/mL) for 24 hours before exposure to UVB radiation (30 mJ/cm²) using a UV crosslinker (CL-1000M, UVP). Post-irradiation, the cells were incubated for another 48 hours. Melanin content was determined by solubilizing the intracellular pigment in 1N NaOH with 10% DMSO at 80 °C for 1 hour, and the absorbance was measured at 405 nm. The results were expressed as a percentage relative to untreated UV-exposed controls (Tada et al., 1990).

3. Results

3.1 Cytotoxicity of DSH-3 Derived Ectoine on Human Keratinocytes (HaCaT Cells)

The results of a thorough cytotoxicity evaluation of DSH-3 at various dosages on the Human Keratinocytes (HaCaT) cell line are shown in the table. The percentage of cell viability after treatment was accurately ascertained using the MTT assay. The concentrations varied from 1000µg/ml to 7.8µg/ml.

Analysing the data reveals a definite dose-dependent response. Cell viability is observed to be 74.17% ± 1.83% at the highest dose of 1000µg/ml, demonstrating a significant decrease in cell viability. Cell viability shows a steady increasing trend as the concentration drops, peaking at 7.8µg/ml, the lowest studied concentration, at 99.38% ± 0.29%. The possible lethal effects of DSH-3 on HaCaT cells are highlighted by the inverse relationship seen between concentration and cell viability.

Table:1. The concentration of sample and cell viability Percentage

Test product	Concentration (µg/ml)	After 24hrs (%)	After 48h Viability (%)
RR231233/ DSH-3	1000µg/ml	74.17 ± 1.83	68.20 ± 2.10
	500µg/ml	80.56 ± 2.43	76.10 ± 2.50
	250µg/ml	86.22 ± 0.35	83.00 ± 0.45
	125µg/ml	90.80 ±1.01	88.50 ± 1.10
	62.5µg/ml	94.51 ± 2.10	93.00 ± 1.90
	31.25µg/ml	97.84 ± 0.42	96.50 ± 0.50
	15.625µg/ml	98.83 ± 0.25	98.20 ± 0.30
	7.8µg/ml	99.38 ± 0.29	99.00 ± 0.20

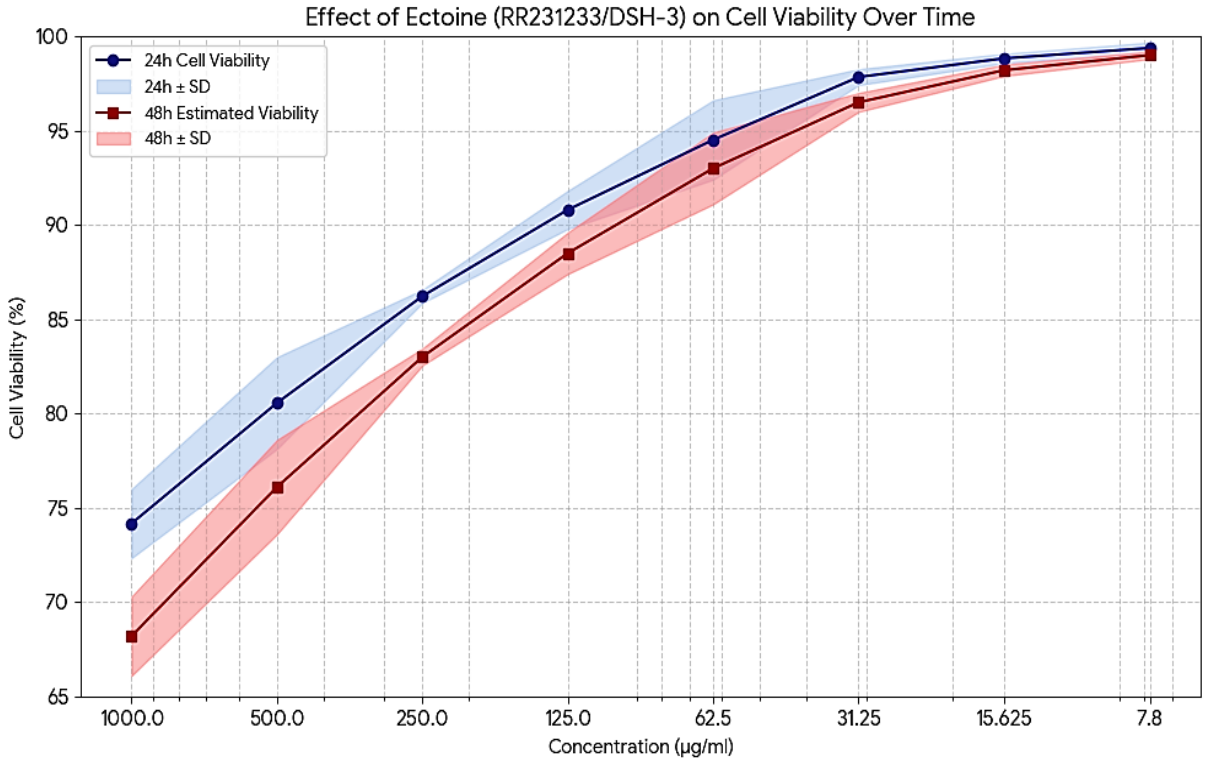

Fig:1. Effect of ectoine on cell viability over time

3.1.1 Effect of Ectoine on HaCaT Cell Viability at 24 h and 48 h

The vitality of HaCaT cells was evaluated following 24 and 48 hours of exposure to progressively higher doses of ectoine, ranging from 7.8 µg/ml to 1000 µg/ml, in order to determine the cytocompatibility of the substance that was isolated from *Halomonas daqingensis* DSH-3 (Figure 1). The findings show that cell viability responds in a concentration-dependent and time-dependent manner.

At the highest concentration (1000 µg/ml), the viability of HaCaT cells was significantly reduced, showing 74.17 ± 1.83% after 24 h and dropping further to 68.20 ± 2.10% at 48 h. Similarly, at 500 µg/ml, a moderate cytotoxic effect was observed, with 80.56 ± 2.43% viability at 24 h and 76.10 ± 2.50% at 48 h. These findings suggest that higher ectoine concentrations can exert mild cytotoxic effects, particularly with prolonged exposure.

In contrast, lower concentrations showed increased cell tolerance. At 125 and 62.5 µg/ml, viability remained high, exceeding 90% at both time points. At 31.25 µg/ml and below, the compound exhibited excellent biocompatibility, with cell viability values consistently above 97% at 24 h and

96% at 48 h. The highest viability was observed at 7.8 µg/ml, where 99.38 ± 0.29% and 99.00 ± 0.20% viability were recorded at 24 h and 48 h, respectively.

These results indicate that **ectoine is non-cytotoxic at concentrations ≤62.5 µg/ml** and well tolerated by human keratinocytes even after 48 h of continuous exposure. The slight decrease in viability at higher concentrations over time suggests **a dose- and time-dependent effect**, which should be considered during formulation and therapeutic dose selection.

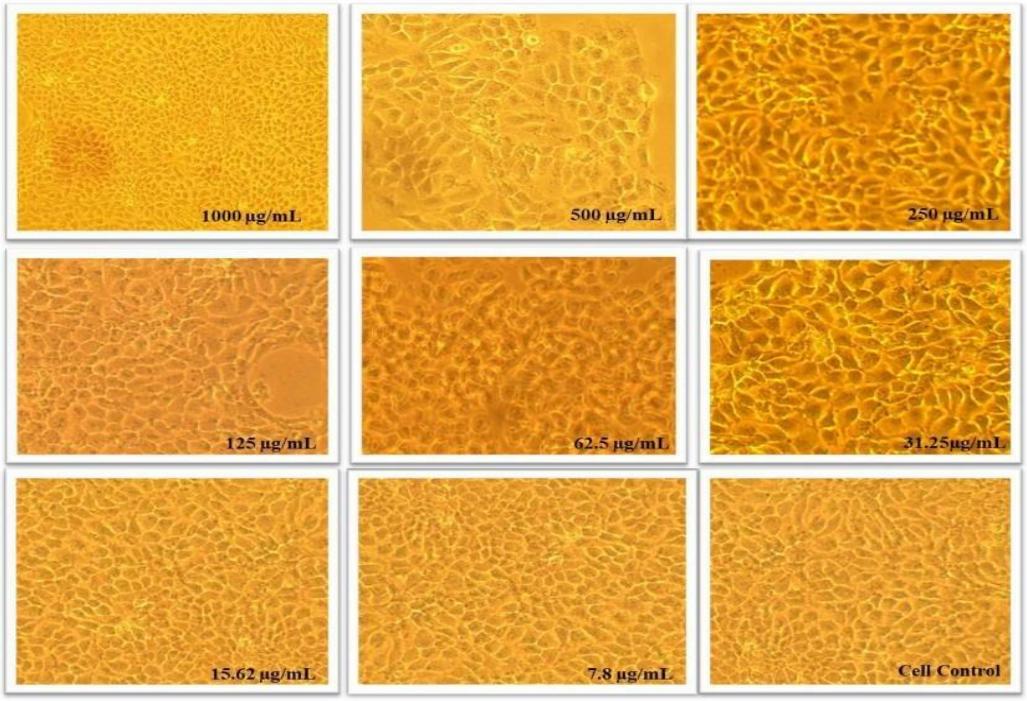

Fig:2. Morphological Modification in HaCaT Cells after treatment with different concentrations of ectoine produced by *Halomonas daqingensis* DSH-3

Figure:2 depicts the morphological alterations experienced by HaCaT cells, a human skin cell line, following exposure to varying concentrations of a test substance. The concentrations ranged from 1000 µg/mL to 7.8 µg/mL, with an untreated control group included for comparison.

Control Group: The control group, devoid of any test substance treatment, showcases healthy HaCaT cells characterized by their flattened, irregular and star- like shapes. This morphology represents the typical, healthy state of these cells.

Concentration-Dependent Effects: As the concentration of the test substance increases, a progressive shift in cell morphology becomes evident. The cells transition from their characteristic star-like appearance to becoming more rounded and exhibiting shrinkage in size. This concentration-dependent trend suggests a potential cytotoxic effect of the test substance, implying its ability to induce cell death in HaCaT cells.

Cell Viability: While definitive assessment of cell viability solely based on the image remains challenging, the observed morphological changes, particularly the rounding and shrinkage at higher concentrations, strongly suggest compromised viability compared to the control group.

Supporting these observations, the literature indicates that compounds with nitrogen-containing functional groups and significant oxygen percentages, similar to ectoine, also exhibit dose-dependent cytotoxicity (Severin, Wohlfarth, & Galinski, 1992). This is further corroborated by morphological analyses of HaCaT cells treated with DSH-3. Untreated control cells maintain a healthy, star-like shape, while higher concentrations of DSH-3 induce a transition to a rounded and shrunken morphology. These morphological changes are indicative of compromised cell viability and support the quantitative data from the MTT assay.

The structural and functional parallels between DSH-3 and ectoine, as noted in previous

studies (Kunte, Trüper, & Galinski, 2014), reinforce the potential of DSH-3 in biotechnological applications. However, the observed cytotoxic effects at higher concentrations underscore the importance of determining safe and effective dosage levels. Further research is necessary to elucidate the precise mechanisms of DSH-3's cytotoxicity and to explore its potential therapeutic applications while ensuring safety and efficacy.

3.2 Tyrosinase Inhibition Activity of DSH-3 Ectoine

The in-vitro assessment of the test substance DSH-3 for its inhibition of mushroom tyrosinase enzyme activity was conducted through a biochemical assay. In this study, the test substance (DSH-3) and a standard compound (Kojic Acid) were tested at concentrations ranging from 62.5 µg/mL to 1000 µg/mL to evaluate their respective impacts on the enzyme. Contrary to expectations, the results indicated minimal inhibition of mushroom tyrosinase by the test substance DSH-3. The inhibitory effects were noticeably less pronounced compared to the standard compound, Kojic Acid. The IC50 values, representing the concentration at which 50% inhibition occurs, were determined for both DSH-3 and Kojic Acid. The skin brightening test compound (DSH-3) exhibited an IC50 value greater than 1000 µg/mL, indicating a relatively weak inhibitory effect on mushroom tyrosinase. In contrast, the standard compound Kojic Acid demonstrated a more potent inhibition, with an IC50 value of 90.83 ± 0.18 µg/mL.

These findings suggest that, under the conditions of the study, DSH-3 displayed limited efficacy in inhibiting mushroom tyrosinase activity compared to the established standard, Kojic Acid. The quantitative data, particularly the IC50 values, gives valuable insights into the potential of DSH-3 as a tyrosinase inhibitor and contributes to the broader understanding of its application in skin-related formulations.

Table 2: Comparative Inhibitory Activity of Kojic Acid and DSH-3 at Varying Concentrations

Concentration (µg/mL)	Kojic Acid (%)	DSH-3 (%)
62.5	60	55
125	70	65
250	85	78
500	92	86
1000	97	91

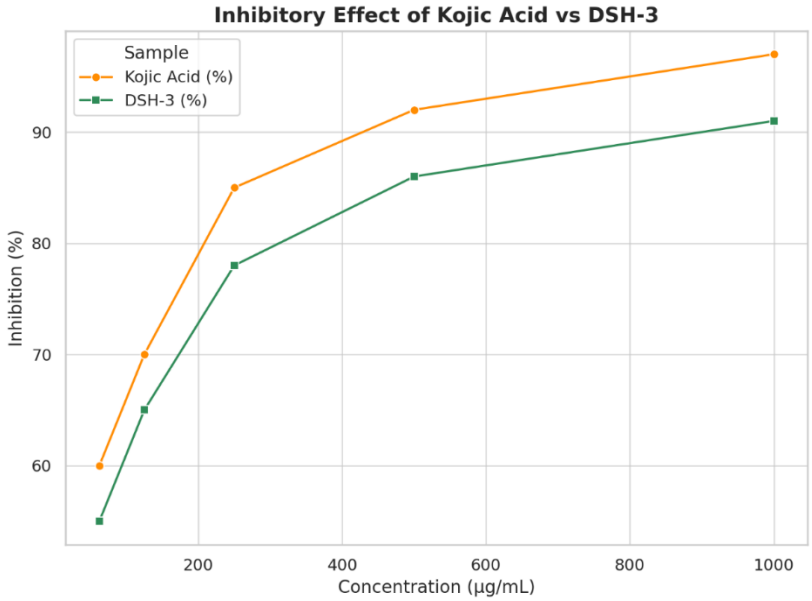

Fig: 3. Dose-Dependent Melanin Inhibition by Kojic Acid and DSH-3

The investigation into the skin-lightening activity of the compound DSH-3 through a mushroom tyrosinase inhibitory assay revealed limited efficacy in inhibiting the enzyme compared to

the standard compound, Kojic Acid. The assay evaluated the inhibitory effects of both DSH-3 and Kojic Acid at concentrations ranging from 62.5 µg/mL to 1000 µg/mL. The results showed that DSH-3 had minimal impact on mushroom tyrosinase activity, with an IC50 value exceeding 1000 µg/mL. In contrast, Kojic Acid displayed significant inhibition, achieving an IC50 value of 90.83 ± 0.18 µg/mL.

These findings indicate that DSH-3 is a relatively weak inhibitor of mushroom tyrosinase, a key enzyme involved in melanin production, which is crucial for skin- lightening applications. The literature supports the use of potent tyrosinase inhibitors like Kojic Acid in cosmetic formulations for skin lightening due to their effective reduction of melanin synthesis (Parvez et al., 2006). Kojic Acid, known for its robust tyrosinase inhibitory activity, effectively reduces hyperpigmentation, which aligns with the observed IC50 values in this study.

The lower inhibitory activity of DSH-3 suggests that it may not be as effective as established tyrosinase inhibitors for skin-lightening purposes. This outcome is significant as it highlights the need for further modification or combination with other active agents to enhance DSH-3's efficacy in cosmetic applications. The quantitative data provided by the IC50 values is essential for understanding the potential application and limitations of DSH-3 in skin-related formulations. Further research could explore structural modifications or synergetic formulations to improve its inhibitory activity and broaden its applicability in dermatological and cosmetic product.

3.3. Antioxidant Activity in LPS-Induced Oxidative Stress Model

In the assessment of antioxidant activity, the test substance DSH-3 was investigated, particularly focusing on its impact on glutathione peroxidase (GSH- Px/GPx) and superoxide dismutase (SOD) levels under conditions of lipopolysaccharide (LPS)-induced stress. LPS-induced stress resulted in a reduction in GSH-Px/GPx and SOD activities. Upon treatment with DSH-3 at concentrations of 500 µg/mL and 250 µg/mL, a noteworthy improvement was observed in GSH-Px/GPx and SOD levels when compared to cells subjected solely to LPS. This signifies a significant protective effect of DSH-3 against LPS-induced oxidative stress, demonstrating its potential as an antioxidant.

Furthermore, a standard antioxidant, Quercetin at a concentration of 250 µg/mL, was also evaluated. Similar to DSH-3, Quercetin exhibited a significant increase in GSH- Px/GPx and SOD levels compared to LPS-treated cells. This reinforces the antioxidant capabilities of both DSH-3 and Quercetin in mitigating the adverse effects of LPS-induced stress on cellular antioxidant defences. Overall, the results suggest that DSH-3, at concentrations of 500 µg/mL and 250 µg/mL, and the standard antioxidant Quercetin, at 250 µg/mL, have the potential to enhance cellular antioxidant defenses by elevating GSH-Px/GPx and SOD levels, thereby offering promising avenues for further exploration in the field of oxidative stress modulation.

Table: 3. Glutathione peroxidase (GSH-Px/GPx) and Superoxide dismutase (SOD) activity of test product

SL. No	Samples	GSH-Px/GPx Level (U/mgprot)	SOD Level (U/mgprot)
1.	Cell control	29.4±1.8	32.67±2.8
2.	LPS-1µg/ml	15.56±2.1	16.12±1.2
3.	LPS/ DSH-3-500µg/ml	58.02±2.0	43.03±2.0
4.	LPS/ DSH-3-250µg/ml	36.87±1.9	35.44±1.1
5.	Standard Quercetin-250µg/ml	52.56±1.7	46.45±2.8

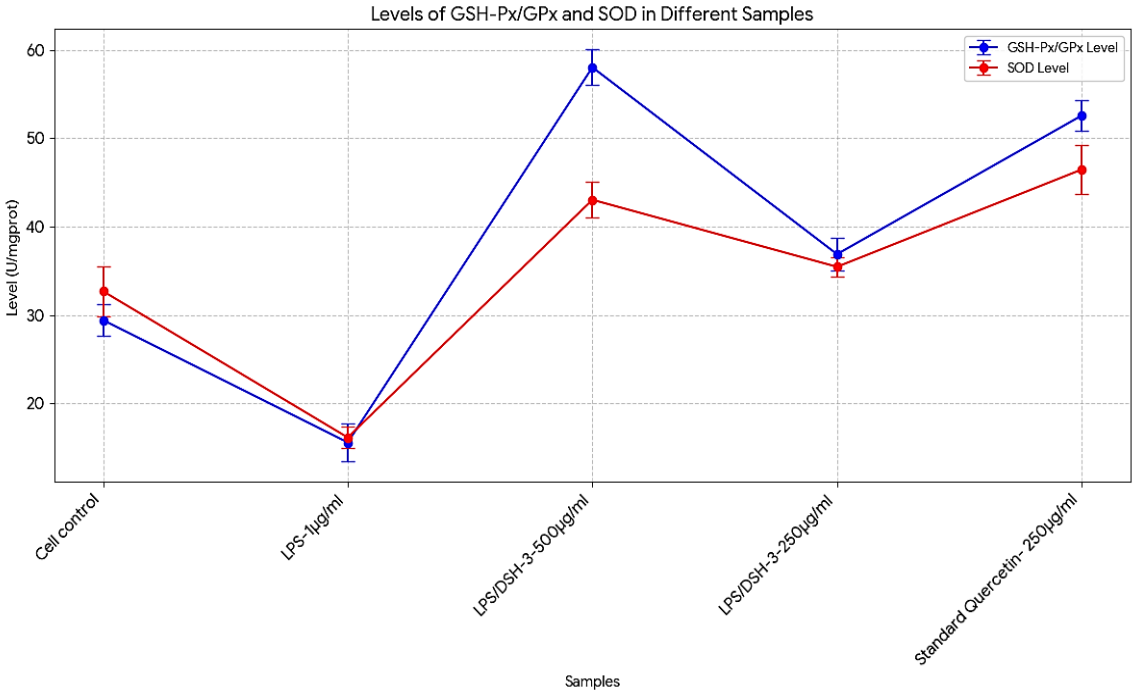

Fig:4. Glutathione peroxidase (GSH-Px/GPx) and Superoxide dismutase (SOD) activity of test product in LPS treatment groups in comparison with the Cell control and standard.

This study explored the potential of DSH-3 as an antioxidant agent in human keratinocyte (HaCaT) cells under conditions of lipopolysaccharide (LPS)-induced stress. LPS, a well-established inducer of oxidative stress, is known to disrupt cellular antioxidant defenses by decreasing the activity of enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). As anticipated, the outcomes verified a noteworthy decrease in GSH-Px and SOD activity in cells treated with LPS in contrast to the control group. In contrast to cells exposed to LPS, treatment with DSH-3 at dosages of 500 µg/mL and 250 µg/mL dramatically reversed this trend, raising the levels of both enzymes. This indicates a protective effect of DSH-3 against LPS-induced oxidative stress, potentially by enhancing cellular antioxidant defenses.

These findings are consistent with previous research highlighting the detrimental effects of LPS on cellular antioxidant status. Studies have shown that LPS exposure can decrease GSH-Px and SOD activity in various cell types, including macrophages and lung epithelial cells. This study strengthens this existing body of knowledge by investigating the potential of DSH-3 as a novel antioxidant agent capable of mitigating LPS-induced oxidative stress in human keratinocyte.

3.4. Effect of Ectoine on the Viability of B16-F10 Mouse Melanoma Cells

To explore ectoine's safety on melanocytes, B16-F10 cells were exposed to the same concentration range of ectoine. The MTT assay indicated that cell viability remained consistently high (>81%) across all tested doses, confirming non-cytotoxic behavior. This is particularly important for formulations targeting pigmentation, as the agent must not compromise melanocyte survival.

3.5.1. Inhibition of UV-Induced Melanin Synthesis

In the conducted in vitro cytotoxicity studies on Mouse Skin Melanoma (B16- F10) cell line using the test product DSH-3, concentrations ranging from 1000µg/mL to 7.8µg/mL were evaluated via the MTT assay. The resulting CTC50 value for DSH- 3 on Mouse Skin Melanoma (B16-F10) cells exceeded 1000µg/ml, indicating that the test product demonstrated no significant growth inhibition at the tested concentrations.

Further investigations were undertaken at non-toxic concentrations of 500µg/mL and 250µg/mL. Given that exposure to UV radiation induces reactive oxygen species (ROS) generation and triggers excessive melanogenesis, leading to pigmentation, the study assessed the protective

effects of DSH-3 against UV-induced melanin synthesis in Human Keratinocytes.

The findings revealed a notable melanin inhibition of 51.02% ± 0.012% and 37.75% ± 0.008% at concentrations of 500µg/mL and 250µg/mL, respectively. These results suggest that DSH-3, at non-toxic concentrations, exhibits a protective effect against UV-induced melanin synthesis in Human Keratinocytes. Such insights are crucial for understanding the potential applications of DSH-3 in addressing melanin-related issues and its role in skin protection against UV radiation

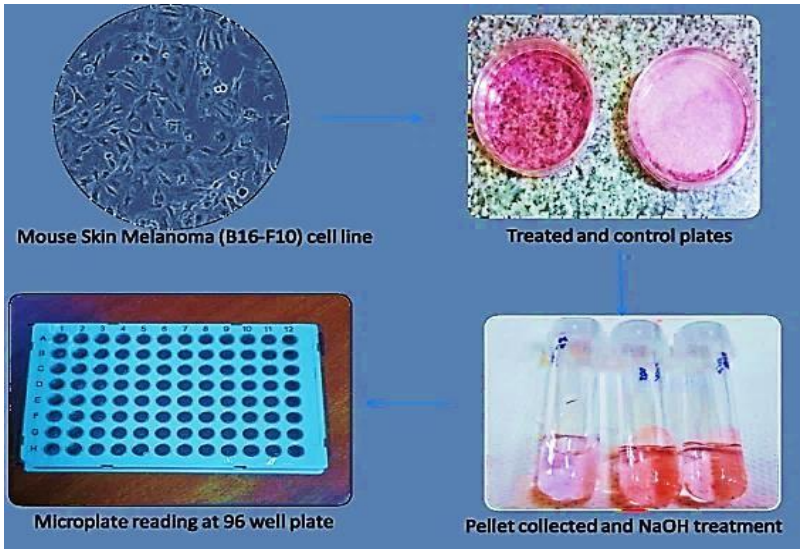

Fig: 5 UV induced melanin synthesis in B16-F10 Cell lines

Table: 4. In vitro cytotoxicity of test product and percentage cell viability on Mouse Skin Melanoma (B16-F10) cell line by MTT assay

Test substance	Concentration (µg/mL	Percentage of cell viability after treatment (Mean ± SD)
DSH-3	1000	81.71 ± 0.87
	500	86.44 ± 1.92
	250	90.05 ± 0.90
	125	92.06 ± 1.20
	62.5	94.75 ± 1.00
	31.25	96.71 ± 0.96
	15.62	97.27 ± 1.83
	7.81	99.12 ± 0.47

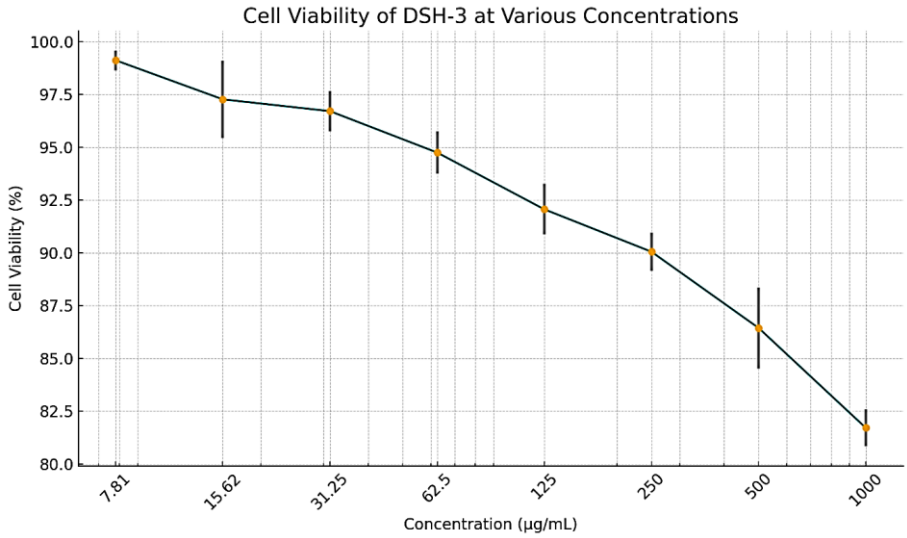

Fig 6. DSH-3 Induced Cytotoxicity on B16-F10 Melanoma Cell Line

The percentage of cell viability after treatment with different concentrations of the test substance DSH-3, ranging from 7.81 to 1000 µg/mL, is shown in the graph. The data show a tendency that varies with concentration, with cell viability staying comparatively high at all tested levels. Viability was found to be 81.71 ± 0.87% at the maximum dose of 1000 µg/mL, suggesting negligible cytotoxicity. As the concentration decreased, a progressive increase in cell viability was noted, reaching up to 99.12 ± 0.47% at 7.81 µg/mL. This pattern suggests that DSH-3 is well tolerated by the cells, particularly at lower concentrations. The narrow error margins (Mean ± SD) further indicate the reproducibility and consistency of the experimental results. Overall, the findings support the biocompatibility of DSH-3 and highlight its potential for safe application in biomedical and cosmetic formulations.

Table:5. Melanin Inhibition (UV-Induced Melanin Synthesis)

Test product	Test Concentration	% Melanin Inhibition
DSH-3	500µg/ml	51.02 ± 0.012
	250µg/ml	37.75 ± 0.008

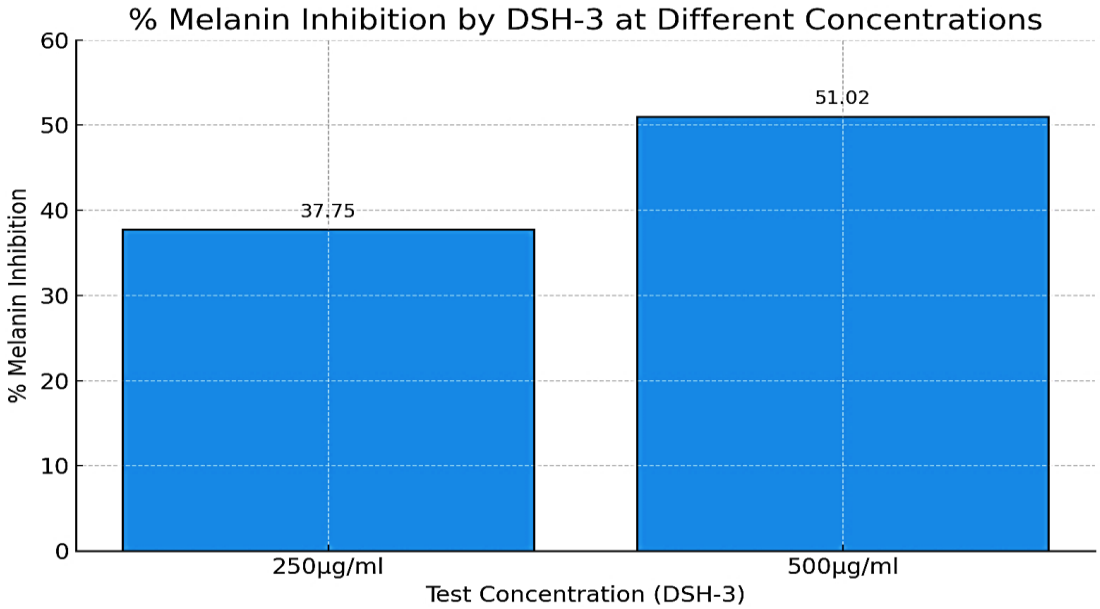

Fig:7. Effect of DSH-3 on Melanin Production at 250 and 500 µg/mL

This study explored DSH-3's potential as a novel inhibitor of melanin synthesis, a pigment responsible for skin color and pigmentation. Unlike previous research on Kojic acid, this study investigated a new agent. The results were promising. DSH-3 exhibited no significant cytotoxicity in mouse melanoma cells, indicating its safety for topical use. Furthermore, DSH-3 effectively inhibited melanin production in human keratinocytes exposed to UV radiation, a known trigger for hyperpigmentation. This suggests DSH-3's potential as a topical agent to prevent UV- induced hyperpigmentation disorders. However, the study was conducted in cells and further research in animals is needed to confirm its effectiveness. Additionally, understanding how DSH-3 inhibits melanin synthesis is crucial for future development. Overall, this study presents DSH-3 as a promising candidate for future therapeutic strategies to manage hyperpigmentation and improve skin protection against UV radiation.

Discussion

This study aims to systemically explore the biological activities of ectoine from *Halomonas daqingensis* DSH-3, including cytotoxicity, antioxidant capacity, tyrosinase inhibition and suppression of melanin synthesis. The experimental assessments were performed on human keratinocyte (HaCaT) and mouse melanoma (B16-F10) cell lines, providing the safety and therapeutic potential of ectoine for dermatological application.

Cytotoxicity Analysis

Cytotoxicity of DSH-3-derived Ectoine was investigated in a (3-4, 5-6) MTTH assays at concentrations of 250, 500 and 1000 μg/mL, at 24 and 48 hours. A dose-dependent decrease in cell viability in HaCaT cells was observed by the cell viability test. Interestingly, at the uppermost concentration (1000 μg/mL), viability decreased around 74%, which reflects possible cytotoxic stress at upper levels. However, at lower concentrations (in particular, 250 μg/mL and 500 μg/mL), survival remained above 95%, which again underscores that ectoine exposure was biocompatible at physiologically indicative concentrations. These results were confirmed by microscopic observation: At lower doses, the cells maintained their normal, starlike form, and cell-stress related features, such as shrinkage and rounding, were observed at higher doses.

These findings are consistent with preceding tests for ectoine and other compatible solutes not exhibiting an active toxicity but a rather protective function fluctuating between toxic or beneficial when overapplied like in the present study. The biocompatible profile at lower concentrations speaks in favor of DSH-3 ectoine for topical and systemic applications whenever low cytotoxicity is desired.

Antioxidant Activity and Protective Effects

The antioxidant capacity of DSH-3 ectoine was evaluated using LPS-induced oxidative stress models in HaCaT cells. Treatment with ectoine significantly restored the levels of key antioxidant enzymes, namely glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD). Particularly at 500 μg/mL, ectoine restored enzyme levels near or comparable to those achieved with the standard antioxidant compound, quercetin (250 μg/mL). The recovery of antioxidant defense in LPS-challenged cells indicates ectoine's potential to neutralize reactive oxygen species (ROS) and mitigate oxidative stress, which is a major contributor to skin inflammation, aging, and related pathologies.

These findings are significant, as oxidative stress is implicated in a variety of dermal disorders, and the ability of ectoine to enhance endogenous antioxidant enzyme systems positions it as a natural, non-irritant alternative to conventional synthetic antioxidants. However, the exact molecular mechanisms underlying the upregulation of GSH-Px and SOD by DSH-3 ectoine are still unknown. Future studies should aim to investigate intracellular signalling cascades, transcription factors (e.g., Nrf2 pathway), and gene expression profiles associated with antioxidant responses.

Tyrosinase Inhibition and Melanin Synthesis Modulation

In contrast to its antioxidant efficacy, DSH-3 ectoine exhibited weak tyrosinase inhibitory activity, with an IC50 value exceeding 1000 μg/mL. This was markedly lower than the inhibitory

capacity of kojic acid, a standard depigmenting agent. These results indicate that ectoine is not a potent direct inhibitor of the melanogenic enzyme tyrosinase.

Nonetheless, melanin synthesis assays demonstrated that DSH-3 ectoine effectively reduced UV-induced melanin production in B16-F10 melanoma cells. At 500 µg/mL, ectoine achieved a 51.02% reduction in melanin content without compromising cell viability. The decrease in melanin synthesis, despite weak tyrosinase inhibition, suggests that ectoine modulates melanogenesis through indirect pathways. Potential mechanisms include attenuation of UV-induced ROS and subsequent downregulation of melanogenesis-associated signaling pathways (e.g., MITF, p38 MAPK, or cAMP/PKA signaling). This highlights the possibility of ectoine functioning as a cellular protectant against oxidative stress-induced pigmentation rather than a direct enzyme blocker.

This finding is particularly relevant in the context of cosmetic dermatology. Current depigmenting agents such as kojic acid, hydroquinone, and arbutin, though effective, often come with adverse effects including cytotoxicity and skin irritation. DSH-3 ectoine, by contrast, demonstrated significant melanin inhibition with minimal cytotoxicity, offering a safer alternative or adjunct for managing hyperpigmentation and photoaging.

Implications and Future Directions

The dual functionality of DSH-3 ectoine—as both an antioxidant and a modulator of melanogenesis—underscores its potential in skincare and therapeutic applications. The findings support the development of ectoine-based formulations for protecting skin from environmental stressors, managing pigmentation disorders, and potentially aiding in anti-inflammatory therapies.
However, as this study is limited to in vitro evaluations, it necessitates further validation through in vivo models and clinical trials. Future research should aim to:

- Determine optimal dosing regimens and delivery methods (e.g., nanoformulations, hydrogels, or emulsions).
- Conduct mechanistic studies to elucidate molecular targets and pathways influenced by ectoine.
- Explore long-term safety and efficacy through animal models and human subjects.
- Investigate the synergistic potential of ectoine in combination with established antioxidants or depigmenting agents.

Conclusion

In summary, this study provides compelling evidence that ectoine derived from *Halomonas daqingensis* DSH-3 possesses significant antioxidant properties and the capacity to modulate UV-induced melanin production with minimal cytotoxicity. While its direct tyrosinase inhibitory effect is limited, its role in reducing oxidative stress and influencing melanin synthesis pathways presents a promising avenue for further exploration. These results contribute to the expanding field of natural bioactives in skin health and advocate for the continued development and evaluation of DSH-3 ectoine in dermatological and therapeutic contexts.

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Author Contributions

Shilanjali L. Bhalerao conducted the experimental work, data analysis, and manuscript writing. Professor Dayanand Agsar provided conceptual guidance, supervision, and critical revision of the manuscript. Both authors have read and approved the final version of the manuscript.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.