https://science.utm.my/procscimath
Volume 29 (2025) 51-59

Clitoria ternatea Extract Mitigates UV-induced Damage in Fibroblast Cells

Siti Nurdinah Ahmad Sukiman[a], Siti Pauliena Mohd Bohari[a,b]*
[a]Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia
[b]Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor
*Corresponding author: pauliena@utm.my

Abstract
Chronic ultraviolet (UV) exposure causes oxidative stress, inflammation, and degradation of skin proteins, leading to aging, impaired healing, and a higher cancer risk. Dermal fibroblasts, key to extracellular matrix production and tissue repair, are highly vulnerable to this damage. *Clitoria ternatea* (butterfly pea) contains anthocyanins, flavonoids, and phenolic acids with strong antioxidant potential, yet its protective effects on UV-damaged fibroblasts remain underexplored. This study optimized an in vitro UV-induced injury model for human fibroblasts and evaluated the cytoprotective effect of *C. ternatea* extract using MTT viability assays and morphology analysis. The optimal UV dose (50 mJ/cm²) induced sublethal stress. Extract treatment improved fibroblast viability and supported recovery compared to UV-only controls. These findings suggest *C. ternatea* as a promising natural photoprotective agent, aligning with the demand for plant-based skincare solutions.
Keywords: *Clitoria ternatea*; ultraviolet radiation; fibroblast; oxidative stress; cytoprotection

Introduction

Ultraviolet (UV) radiation is a significant environmental factor that causes oxidative stress, inflammation, and cellular imbalance in the skin, leading to photoaging, impaired wound healing, and an increased risk of cancer. UVA (320–400 nm) and UVB (280–320 nm), the primary components reaching the Earth's surface, induce long-term damage through the generation of reactive oxygen species (ROS), which degrade collagen and elastin —the key structural proteins in the skin (Calvo et al., 2024). Fibroblasts, the primary dermal cells responsible for synthesizing the extracellular matrix (ECM) and facilitating tissue repair (Diller & Tabor, 2022), are highly susceptible to UV-induced oxidative damage, leading to reduced proliferation and delayed wound healing.

Natural antioxidants are generally attracting researchers for skin protection studies. *Clitoria ternatea* (butterfly pea), on the other hand, is also gaining attention due to its rich anthocyanin, flavonoid, and phenolic acid content. These compounds have a strong free radical scavenging capacity, protect cellular structures, and support fibroblast proliferation and migration (Zakaria et al., 2018). A previous study shows that *C. ternatea* extract can reduce oxidative stress, inhibit matrix metalloproteinases, and promote wound closure (Vidana Gamage et al., 2021). However, its specific protective effects on UV-damaged fibroblasts need further warrants.

This study aims to optimize UV radiation conditions to induce sublethal stress in human fibroblasts and evaluate the effect of *C. ternatea* extract on cell viability and morphology using in vitro assays. The work provides preliminary insights into the potential of *C. ternatea* as a natural photoprotective agent for dermatological applications.

.

Materials

The human fibroblast cell line (HSF) was obtained from previously established laboratory stock. Dried *Clitoria ternatea* flowers were purchased from Herbal Remedies (Malaysia). Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were sourced from Gibco, Thermo Fisher Scientific (USA). Phosphate-buffered saline (PBS) was purchased from Takara Bio Inc. (Japan), while trypsin-EDTA, dimethyl sulfoxide (DMSO), MTT reagent, and 1N hydrochloric acid (HCl) in isopropanol were obtained from Sigma-Aldrich (USA). The main equipment used included an analytical balance (JOANLAB, China), a UVC lamp (Cole-Parmer, USA), a biological safety cabinet (Esco Lifesciences, Singapore), a CO_2 incubator (Bluepard, China), a microplate reader (BioTek Instruments, USA), a rotary evaporator, and a freeze-dryer.

Methods

Preparation of Clitoria ternatea Extract

The extraction method employed in this study was adapted from previously published procedures by Srichaikul (2018) and Jeyaraj et al. (2022), where Dried C. ternatea flowers were ground into a fine powder and then extracted with 70% ethanol (1:10 w/v) for 3 days. The mixture underwent 45 min of ultrasonication at 25°C, followed by filtration (0.45 µm). Solvent was removed using a rotary evaporator (40°C), and the extract was freeze-dried for 48 h. The dried extract was stored at −20°C until further use.

Fibroblast Cell Culture

Human skin fibroblast (HSF) cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2 and subcultured upon reaching 80–90% confluency, with media changes performed every 2–3 days under aseptic conditions.

Optimization of UV Radiation

The optimization of UV radiation was performed based on a study published by Cheng et al. (2023), with some modifications. Cells were seeded in 12-well plates (~1×10^5 cells/well) and incubated overnight. Two UV doses (20 mJ/cm² and 50 mJ/cm²) were tested using a UVC lamp (1 mW/cm²; 1 s exposure = 1 mJ/cm²; 10 cm distance). Morphology and viability were assessed at 24 h and 48 h. The dose causing 30–50% viability reduction (50 mJ/cm²) was selected for subsequent experiments.

Cytotoxicity Assay

Cytotoxicity activity of the extracts was performed according to the published method by Rajput and Bithel (2022), with some modifications. Cells were seeded (~1×10^5 cells/well) and allowed to attach overnight. After UV exposure (50 mJ/cm²), cells were treated with *C. ternatea* extract at concentrations of 15 µg/mL or 25 µg/mL for 72 hours. Morphology was monitored at 24, 48, and 72 h. Cell viability was assessed using the MTT assay: 100 µL of MTT solution (5 mg/mL) was added for 4 h, followed by the dissolution of formazan crystals in 200 µL HCl-isopropanol. Absorbance was read at 570 nm using a microplate reader. Cell viability (%) was calculated relative to untreated controls.

Results and discussion

Extraction of Clitoria ternatea

Table 1 presents the extraction yields of *C. ternatea* from two independent batches, prepared separately on different days using the same extraction protocol. Although both batches began with an equal total volume (800 mL), batch 2 retained a higher volume after filtration (650 mL) compared to batch 1 (400 mL), indicating possible differences in filtration efficiency or particulate load. However, batch 1 exhibited a higher final freeze-dried weight (7.7428 g) than batch 2 (6.8124 g), suggesting variability in extract concentration or solvent removal efficiency. These differences highlight the importance of batch-to-batch consistency in extraction procedures.

Table 1: *Clitoria ternatea* extract volumes and weights obtained from first and second extraction.

Extract	Total Volume (mL)	Volume After Filtration (mL)	Weight After Rotary Evaporatory (g)	Weight After Freeze-Dry (g)
Batch 1	800	400	9.7704	7.7428
Batch 2	800	650	10.0685	6.8124

Optimization of UV Radiation

Table 2 presents the morphological assessment of human fibroblast cells maintained under standard culture conditions in the control wells (well 4: B4 and C4) from May 15 to 18. These wells served as the non-irradiated group (no UV exposure) to evaluate normal cell growth and morphology. Throughout the observation period, the cells consistently displayed typical fibroblast morphology, which is spindle-shaped, adherent monolayers with increasing confluency over time. No signs of cellular stress, detachment, or contamination were observed, thereby validating the suitability of the culture conditions and providing a reliable baseline for comparison with UV-treated groups.

Table 2: Observation of cell morphology in control wells (well B4 and C4) from 15-18 May.

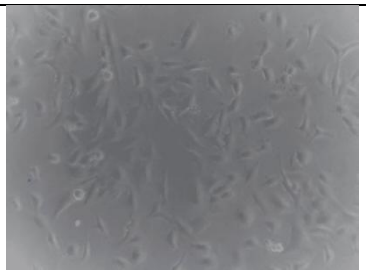
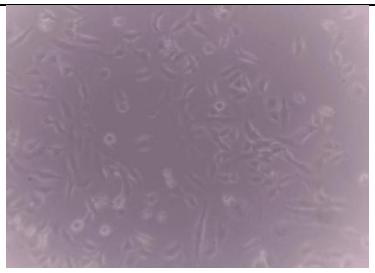
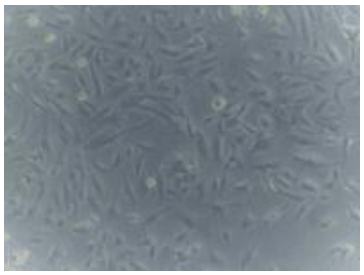
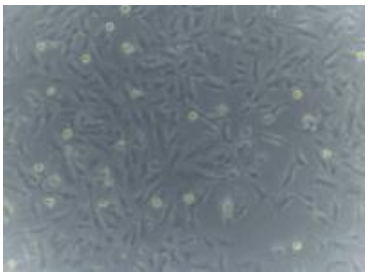
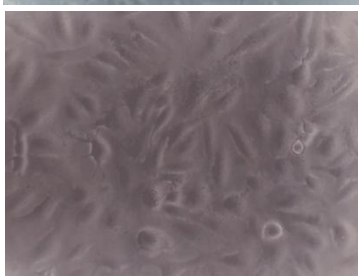
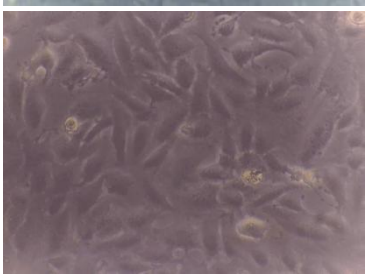
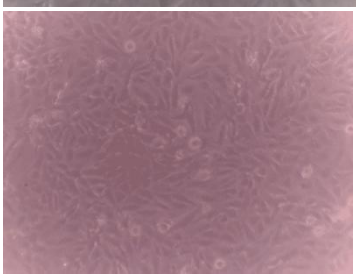
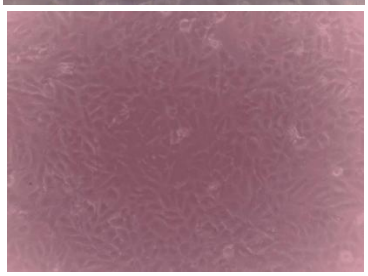
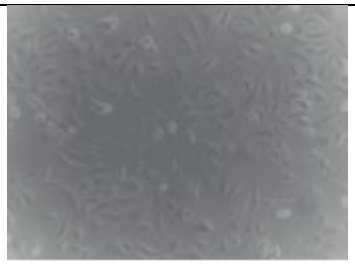
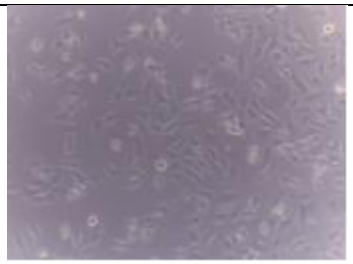
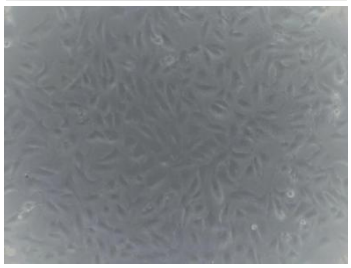
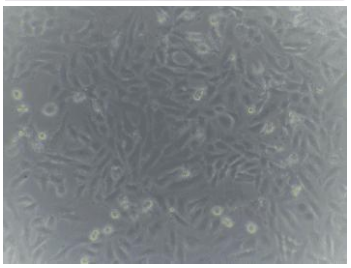
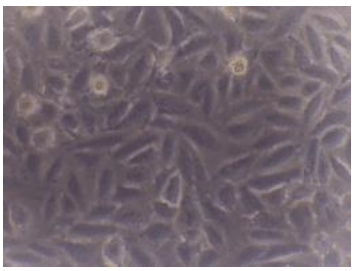
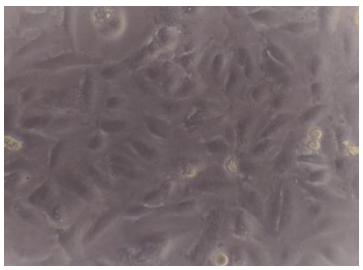
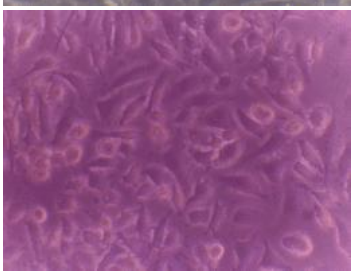
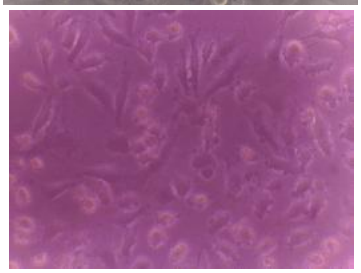
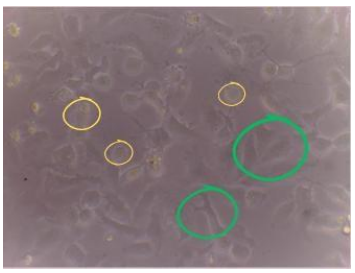
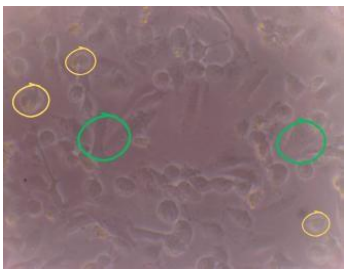
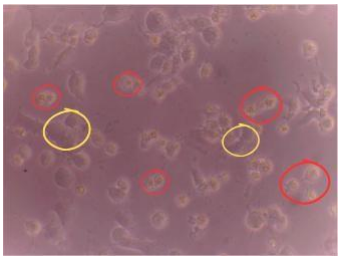
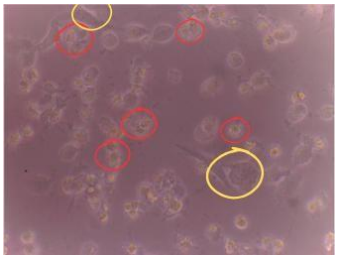
Well 4 (Control)	B4	C4
15 May		
16 May		
17 May		
18 May		

Table 3 summarizes the morphological observations of human fibroblast cells in well 1 (B1 and C1) exposed to UV dose 1 at 20 mJ/cm². Prior to UV exposure, the cells exhibited typical spindle-shaped morphology with progressive growth from 24 to 48 hours post-seeding. The cells were

assessed before UV exposure and maintained healthy morphology. Immediately after UV exposure, a slight alteration in morphology was noted. After 24 and 48 hours post-irradiation, observable changes included minor cell shrinkage and reduced density, indicating the early signs of stress response without complete cytotoxicity. As illustrated in the table, the cellular response following treatment was categorized based on both cell viability and morphological characteristics. Green-labelled cells represent normal, healthy fibroblasts with intact morphology and robust metabolic activity. Yellow-labelled cells indicate surviving but physiologically stressed cells, showing minor morphological alterations or reduced viability. Red-labelled cells denote defective or damaged fibroblasts, exhibiting features consistent with apoptosis or severe cellular stress. This colour-coding scheme provides a clear visual representation of the treatment's effects on fibroblast health and integrity. These findings support the role of 20 mJ/cm² as a low-level stressor suitable for optimization studies assessing sublethal cellular responses.

Table 3: Morphological observation of fibroblast cells in well 1 (B1 and C1) before and after exposure to 20 mJ/cm² UV dose.

Well 1 (20 mJ/cm²)	**B1**	**C1**
After 24 hours of seeding		
After 48 hours of seeding		
Before UV		
Right after UV		

After 24 hours of UV	 Green: normal cells Yellow: surviving cells	 Green: normal cells Yellow: surviving cells
After 48 hours of UV	 Yellow: surviving cells Red: defective cells	 Yellow: surviving cells Red: defective cells

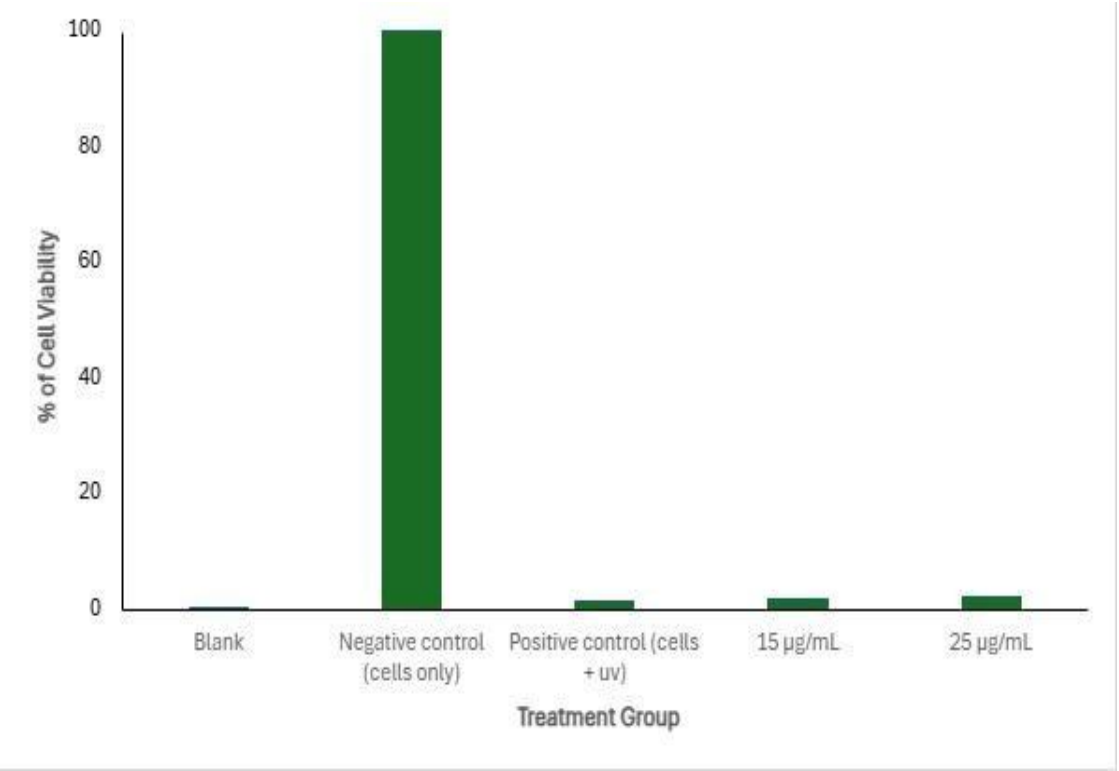
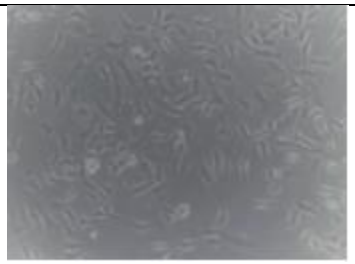
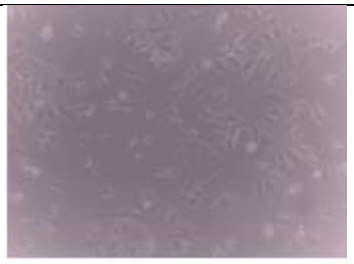
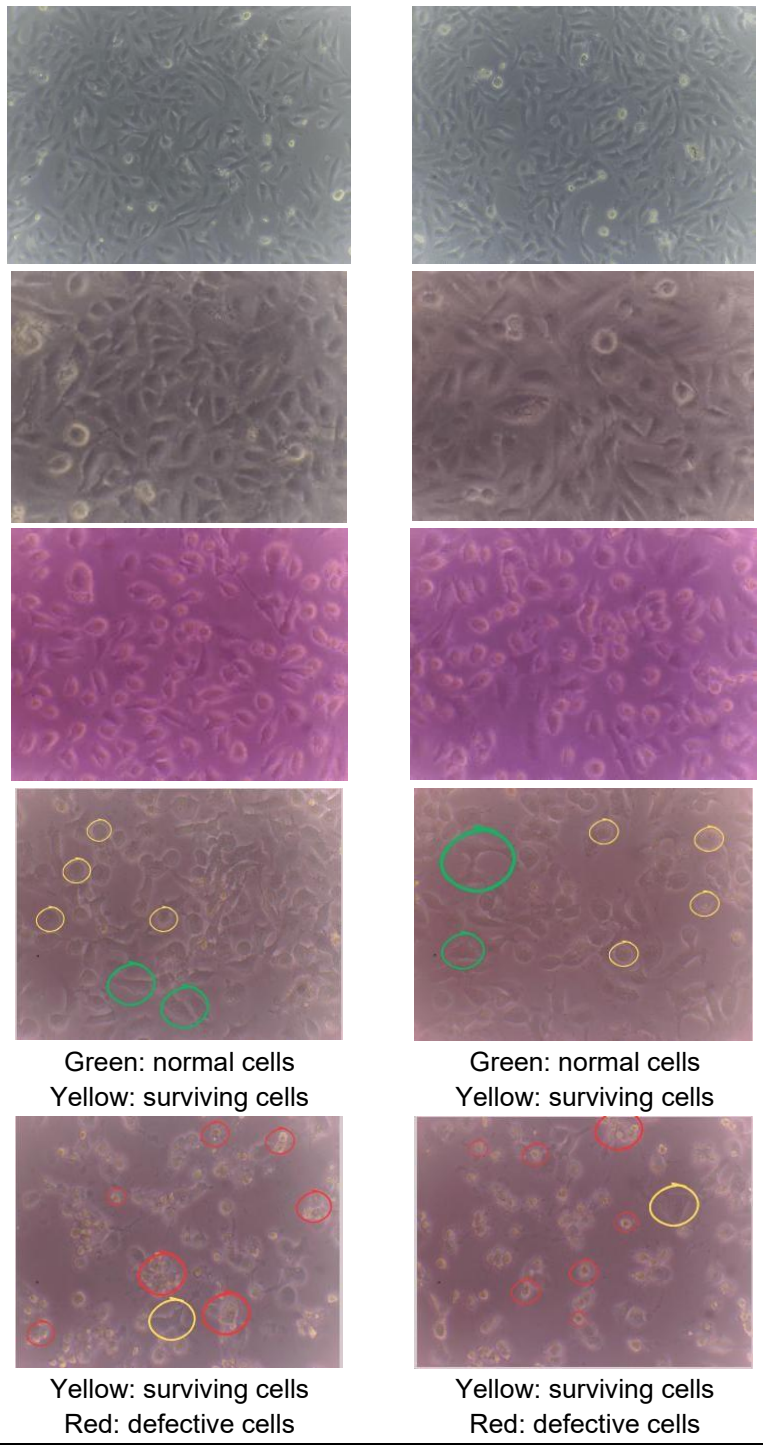


Figure 1 Cell viability (%) graph of UV-exposed fibroblasts treated with *Clitoria ternatea* extract.

Table 4 presents the sequential morphological changes observed in human fibroblast cells seeded in well 2 (B2 and C2), which were exposed to a higher UV radiation dose of 50 mJ/cm². Prior to irradiation, cells displayed healthy growth, with increasing confluency and typical fibroblast morphology. Immediate post-irradiation assessment revealed visible morphological alterations, including cell shrinkage and partial detachment. At 24 and 48 hours post-UV exposure, more pronounced cytotoxic effects were evident, such as reduced cell density, rounded morphology, and disrupted monolayer integrity. These observations suggest that a 50 mJ/cm² UV dose induces significant cellular stress and viability loss, supporting its identification as the optimal dose for inducing measurable sublethal damage in further cell viability testing.

Table 4: Morphological observation of fibroblast cells in well 2 (B2 and C2) before and after exposure to 50 mJ/cm² UV dose.

Well 1 (50 mJ/cm²)	**B2**	**C2**
After 24 hours of seeding		



Microscopic evaluation of fibroblast morphology was conducted at four time points: before UV exposure, immediately after exposure, and at 24 h and 48 h post-exposure. Prior to UV irradiation, cells exhibited healthy elongated, spindle-shaped morphology with clear boundaries and strong adherence, consistent with a healthy fibroblast phenotype as previously described by Yao et al. (2022). At 20 mJ/cm², fibroblasts showed mild, transient changes immediately after exposure, including slight cytoplasmic granularity and altered shape. Most cells regained normal morphology and proliferation within 24 h, indicating reversible, sublethal stress. This aligns with previous findings that low-intensity UV causes limited oxidative stress and reversible effects in dermal fibroblasts (Kim et al., 2019).

In contrast, 50 mJ/cm² UV exposure caused pronounced morphological alterations. Immediately after irradiation, many cells became rounded, less adherent, and exhibited dense

cytoplasm, suggesting stress possibly activating apoptotic or autophagic pathways. At 24 h, partial recovery was seen, but many cells retained abnormal morphology with reduced confluency. After 48 hours, these abnormalities persisted, indicating sustained cytotoxicity consistent with higher UV-induced oxidative stress, mitochondrial dysfunction, DNA damage, and inhibition of proliferation (Lee et al., 2022).

The dose-dependent differences confirm that 50 mJ/cm² induces consistent sublethal damage, making it an optimal model for evaluating *the protective potential of C. ternatea extract*. This UV level produced measurable oxidative stress without complete cell death, providing a therapeutic window for antioxidant and reparative interventions. Given *C. ternatea*'s rich content in anthocyanin, flavonoids, and phenolic acids (Ong et al., 2022), this model enables a meaningful assessment of its antioxidant, anti-inflammatory, and DNA-protective properties (Kim et al., 2019).

Cytotoxicity Assay

MTT assay results showed that UV exposure significantly reduced fibroblast viability, evident from the reduced formation of purple formazan crystals and lower absorbance readings, confirming successful induction of UV-related cellular damage. The negative control (untreated cells) maintained 100% viability, whereas the UV-only group dropped sharply to 1.24%. Treatment with *C. ternatea* extract at 15 µg/mL and 25 µg/mL slightly improved viability to 1.64% and 1.70%, respectively, but remained below 2%, indicating minimal cytoprotective effect under the tested conditions (Figure 1).

These findings contrast with in vivo studies where *C. ternatea* extract has shown significant photoprotective and reparative effects, likely due to systemic factors, optimal dosing, and complex extracellular interactions absent in cell culture. For example, topical application of *C. ternatea* cream at 2.5%–5% in UVB-exposed rat skin increased fibroblast counts and improved tissue repair, attributed to its high flavonoid and anthocyanin content with antioxidant and anti-inflammatory properties (Nursyafillah et al., 2025). The discrepancy may be due to differences in concentration, treatment duration, and the acute nature of the in vitro assay, which captures only short-term responses. In vivo systems benefit from sustained antioxidant activity, enhanced collagen synthesis, and extracellular matrix interactions not replicated in vitro.

Despite the limited effect observed, the established UV-induced fibroblast injury model at 50 mJ/cm² provides a robust and reproducible platform for further research. Future studies should test broader concentration ranges, longer treatment periods, and additional endpoints such as ROS quantification, DNA damage assays, apoptosis markers, and collagen synthesis evaluation. Such optimizations will help clarify *C. ternatea*'s potential as a natural photoprotective agent.

Conclusion

This study established a reproducible in vitro model of UV-induced fibroblast injury, identifying 50 mJ/cm² as an optimal sublethal dose. This exposure caused clear morphological changes and reduced viability, providing a reliable platform for cytoprotection testing. At 15 µg/mL and 25 µg/mL, *Clitoria ternatea* extract slightly increased cell viability compared to UV-only controls (remaining below 2%), indicating limited acute protection under the tested conditions. The weaker effect compared to in vivo studies may be due to differences in model systems, extract concentration, treatment duration, and the absence of physiological factors.

Future work should explore a wider concentration range and alternative formulations, as well as preventive (pre-UV) treatments to assess prophylactic potential. Additional endpoints such as ROS levels, DNA damage, apoptosis, and proliferation rates would clarify underlying mechanisms. Incorporating in vivo or co-culture models will help bridge the gap between in vitro and real biological responses. Consistent extraction methods, batch quality control, and robust statistical analyses are recommended to enhance reproducibility and strengthen conclusions. These steps will support the development of *C. ternatea* as a potential photoprotective agent in dermatological applications.

Acknowledgement

The authors would like to express their gratitude to the Faculty of Science Laboratory Management, Tissue Engineering Laboratory, TEL, and the Institute of Bioproduct Development, IBD (N22),

Universiti Teknologi Malaysia, Skudai, for providing the necessary laboratory facilities and support under UTM Fundamental Grant (23H50).

References

Calvo, M. J., Navarro, C., Durán, P., Galan-Freyle, N. J., Hernández, L. A. P., Pacheco-Londoño, L. C., Castelanich, D., Bermúdez, V., & Chacin, M. (2024). Antioxidants in photoaging: From molecular insights to clinical applications. *International Journal of Molecular Sciences, 25*(4), 2403.

Cheng, Y., Xia, Q., Lu, Z., Luan, X., Fan, L., Wang, Z., & Luo, D. (2023). Maslinic acid attenuates UVB-induced oxidative damage in HFF-1 cells. *Journal of Cosmetic Dermatology, 22*(8), 2352–2360.

Diller, R. B., & Tabor, A. J. (2022). The role of the extracellular matrix (ECM) in wound healing: A review. *Biomimetics, 7*(3), 87.

Jeyaraj, E. J., Lim, Y. Y., & Choo, W. S. (2022). Antioxidant, cytotoxic, and antibacterial activities of *Clitoria ternatea* flower extracts and anthocyanin-rich fraction. *Scientific Reports, 12*(1), 19146.

Kim, M., Kim, S. M., & Kang, S. W. (2019). Ultraviolet B-induced oxidative stress, cell death and DNA damage in human dermal fibroblasts: Protective effect of antioxidants. *Journal of Dermatological Science, 93*(2), 110–117.

Lee, H. E., Lee, J. Y., & Kim, Y. (2022). Protective effects of natural compounds against UVB-induced skin damage in human fibroblasts. *International Journal of Molecular Sciences, 23*(3), 1457.

Nursyafillah, R. N., Puruhito, B., Afriliana, L., Istiadi, H., & Damayanti, G. S. (2025). Potential of butterfly pea cream (*Clitoria ternatea*) in skin regeneration by increasing the number of fibroblasts and collagen thickness. *Jurnal Kedokteran Diponegoro, 14*(1), 44–49.

Ong, S. L., Khoo, L. W., & Tan, C. P. (2022). *Clitoria ternatea* (butterfly pea) extract protects human dermal fibroblasts from oxidative stress: Potential for cosmeceutical application. *Molecules, 27*(16), 5122.

Rajput, M., & Bithel, N. (2022). Phytochemical characterization and evaluation of antioxidant, antimicrobial, antibiofilm and anticancer activities of ethyl acetate seed extract of *Hydnocarpus laurifolia*. *3 Biotech, 12*(9), 401.

Srichaikul, B. (2018). Ultrasonication extraction, bioactivity, antioxidant activity, total flavonoid, total phenolic and antioxidant of *Clitoria ternatea* Linn flower extract for anti-aging drinks. *Pharmacognosy Magazine, 14*(56), 322–328.

Sukiman, S. N. A., & Bohari, S. P. M. (2025). *Clitoria ternatea* extract mitigates UV-induced damage in fibroblast cells. *Proceedings of Science and Mathematics, 29,* 51–60.

Vidana Gamage, G. C., Lim, Y. Y., & Choo, W. S. (2021). Anthocyanins from *Clitoria ternatea* flower: Biosynthesis, extraction, stability, antioxidant activity, and applications. *Frontiers in Plant Science, 12,* 792303.

Yao, X., Li, H., Chen, L., & Tan, L. P. (2022). UV-induced senescence of human dermal fibroblasts restrained by low-stiffness matrix by inhibiting NF-κB activation. *Engineered Regeneration, 3*(4), 365–373.

Zakaria, N., Okello, E., Howes, M., Birch-Machin, M., & Bowman, A. (2018). In vitro protective effect of an aqueous extract of *Clitoria ternatea* L. flower against hydrogen peroxide-induced cytotoxicity and UV-induced mtDNA damage in human keratinocytes. *Phytotherapy Research, 32*(6), 1064–1072.