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In Vivo Study of Telang Cream on Interleukin (IL)-6, Epidermal Growth Factor (EGF), and Wound Reepithelialization

Studi In-Vivo Krim Telang terhadap Interleukin (IL)-6, Epidermal Growth Factor (EGF), dan Reepitelisasi Luka

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ABSTRACT

Background: Povidone iodine is widely used in wound healing but can cause irritation and cellular toxicity. Clitoria ternatea L. contains compounds such as rutin, quercetin 3-O-rhamnoside, ternatin, and anthocyanins that offer potential as a natural alternative for wound healing. This study specifically utilized the anthocyanin compounds present in the extract.

Objective: This study aimed to evaluate the effects of Clitoria ternatea L. extract cream on Interleukin-6 expression, Epidermal Growth Factor, and re-epithelialization in incision wounds of Sprague Dawley rats.

Methods: This in-vivo research employed a post-test only control design with five treatment groups, including unwounded control (healthy), povidone iodine treatment, and Clitoria ternatea L. extract cream (5%, 10%, 15%). IL-6 and EGF analysis was performed using immunohistochemistry, while re-epithelialization was assessed through hematoxylin-eosin staining.

Results: Clitoria ternatea L. extract enhanced IL-6 and EGF expression, with the highest expression observed at 10% concentration. Re-epithelialization occurred more rapidly in the treatment groups compared to both positive and negative controls.

Conclusion: The 10% Clitoria ternatea L. extract cream demonstrated comparable effectiveness to povidone iodine in healing incision wounds.

Keywords: Clitoria ternatea L; IL-6; EGF; Re-epithelialization; Wound healing

ABSTRAK

Latar Belakang *Povidone iodine banyak digunakan dalam penyembuhan luka, tetapi dapat menyebabkan iritasi dan toksisitas seluler. Clitoria ternatea L. memiliki potensi kandungan senyawa seperti rutin, quercetin 3-O-rhamnoside, ternatin, dan antosianin sebagai alternatif alami dalam penyembuhan lukam pada penelitian ini ekstrak yang digunakan menggunakan senyawa antosianin yang terkandung didalamnya.*

Tujuan*: Penelitian bertujuan menilai efek krim ekstrak Clitoria ternatea L. terhadap ekspresi Interleukin-6, Epidermal growth factor, dan re-epitelisasi pada luka insisi tikus Sprague dawley.*

Metode: *Penelitian in-vivo dengan desain kontrol post-test only pada lima kelompok perlakuan, termasuk kontrol tanpa luka (sehat), povidone iodine serta krim ekstrak Clitoria ternatea L. (5%, 10%, 15%). Analisis IL-6 dan EGF menggunakan imunohistokimia, sementara re-epitelisasi dinilai dengan pewarnaan hematoksilin-eosin.*

Hasil *Ekstrak Clitoria ternatea L. meningkatkan ekspresi IL-6 dan EGF, dengan ekspresi tertinggi pada konsentrasi 10%. Re-epitelisasi lebih cepat pada kelompok perlakuan dibandingkan kontrol positif maupun negatif.*

Kesimpulan: *Krim ekstrak Clitoria ternatea L. 10% memiliki efektivitas yang sebanding dengan povidone iodine dalam penyembuhan luka insisi.*

Kata Kunci: *Clitoria ternatea L; IL-6; EGF; Re-epitelisasi; Penyembuhan luka*

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INTRODUCTION

Wound healing involves complex interactions between cells, inflammatory mediators, growth factors, and extracellular matrix components (Johnson *et al.*, 2020; Puspitasari, Saputri and Winahyu, 2023; Wulandari and Oktariana, 2024). Interleukin-6 (IL-6) functions as a pro-inflammatory mediator that facilitates leukocyte recruitment to the wound site, while Epidermal Growth Factor (EGF) promotes the proliferation of epithelial cells and fibroblasts (Johnson et al., 2020; Nabila Latifa Hafizsha et al., 2021). Although povidone-iodine is widely utilized in wound management, its cytotoxic properties may induce irritation and impede re-epithelialization (Amfotis, Suarni and Arpiwi, 2022). Consequently, there is growing interest in natural alternatives such as Clitoria ternatea L. extract, which demonstrates anti-inflammatory, antioxidant, and tissue regeneration-promoting properties (Suherlan, Muhammad Fakih and Herawati Effendi, 2021).

According to Irenesia, Islami and Utami (2023), 10% povidone-iodine is a broad-spectrum antiseptic commonly employed in wound care due to its bacteriostatic (640 µg/ml) and bactericidal (960 µg/ml) effects, which effectively inhibit pathogenic microorganisms and reduce sepsis in burn wounds, thereby accelerating the healing process. However, prolonged application can result in adverse effects, including fibroblast toxicity, cutaneous irritation, allergic reactions, and cytotoxicity to adjacent tissues. Multiple investigations of Clitoria ternatea L. have demonstrated its therapeutic potential Zona et al. (2021) evaluated the anti-inflammatory activity of Clitoria ternatea L. bioactive compounds against cyclooxygenase-2 (COX-2) enzyme using Quantitative Structure-Activity Relationship (QSAR) and in silico methodologies. Their findings revealed that compounds such as rutin, quercetin 3-O-rhamnoside, ternatin, and anthocyanin exhibit superior anti-inflammatory efficacy compared to aspirin as a reference inhibitor (Putri, Fakih and Suarantika, 2024).

Puspitasari, et al. (2022) investigated the efficacy of Clitoria ternatea L. extract cream at concentrations of 5%, 10%, and 15% in promoting incisional wound healing in Wistar rats, using 10% povidone-iodine as a comparator. The 15% concentration demonstrated optimal outcomes, achieving complete wound closure by day 7 (Puspitasari et al., 2022). Another study examining the effects of Clitoria ternatea L. extract cream on incisional wounds in rats reported that both the 15% concentration and povidone-iodine facilitated complete healing by day 7, while the 5% and 10% concentrations achieved wound closure by day 8, and the negative control by day 12. Additionally, Fatmawati (2022) evaluated the photoprotective effects of Clitoria ternatea L. extract on erythema and pigmentation in rabbit skin following ultraviolet B (UVB) exposure. Results indicated that the 10% formulation exhibited maximal efficacy as a sunscreen agent.

To date, studies specifically examining the effects of Clitoria ternatea L. extract on IL-6 expression, EGF production, and re-epithelialization in incisional wound healing remain absent from the scientific literature. Previous research has explored the effects of alternative plant extracts on these parameters, such as the investigation of bay leaf (Syzygium polyanthum) extract gel on IL-10 and IL-6 gene expression in traumatic ulcers of Wistar rats, which demonstrated that the extract reduced IL-6 levels while elevating IL-10 levels, thereby expediting the wound healing process (Shafia, 2023; Adaninggar, 2024). Despite the existence of comparable studies utilizing different plant extracts, research specifically focused on elucidating the effects of Clitoria ternatea L. extract on

IL-6 expression, EGF production, and re-epithelialization in incisional wound healing represents a novel contribution to the scientific literature that warrants further exploration.

The present study aims to evaluate the effects of Clitoria ternatea L. extract cream on IL-6 expression, EGF production, and re-epithelialization in incisional wounds in Sprague-Dawley rats, with the objective of identifying a non-irritating and non-toxic alternative topical agent for wound healing.

MATERIALS AND METHODS

A. Research Design

This study employed an in vivo experimental approach with a Post-Test Only Control Group Design to evaluate the efficacy of Clitoria ternatea L. extract cream on incision wounds in male Sprague Dawley rats (Putri, Fakih and Suarantika, 2024). The experimental protocol received approval from the Health Research Ethics Committee of YARSI University (134/KEP-UY/EA.10/VI/2024).

B. Population and Sample

The study population comprised male Sprague Dawley rats that met the following inclusion criteria: age 2-3 months, body weight 200-250 grams, and exhibiting healthy and active behavior during the adaptation period. Rats displaying signs of illness during adaptation or presenting anatomical abnormalities were excluded from the study.

Sample size determination utilized the *Federer* formula: (t-1)(n-1)≥15. With 5 treatment groups, a minimum of five replicates per group was required, yielding 25 rats per assessment point. As evaluations were conducted on days 3, 7, and 15, the total sample comprised 75 rats.

The experimental groups were classified into 5 categories:

1. Negative control (healthy rats without wound or treatment)
2. Positive control (wounded rats treated with povidone iodine)
3. Clitoria ternatea L. 5% extract cream treatment
4. Clitoria ternatea L. 10% extract cream treatment
5. Clitoria ternatea L. 15% extract cream treatment

Data collection involved histological and immunohistochemical analyses to assess IL-6 expression, EGF expression, and cutaneous re-epithelialization. This methodological approach aligns with previous investigations demonstrating the significance of these parameters in wound healing processes (Sarmila *et al.*, 2021; Herdiani, Pramasari and Purnamasari, 2022).

C. Data Collection Procedures

This investigation utilized primary data obtained directly through experimentation. Microscopic observations were conducted using a light microscope across three distinct fields of view, with systematic lateral shifts to ensure comprehensive assessment.

1. **Sample characteristics:** Male Sprague Dawley rats aged 2-3 months weighing 200-250 grams were utilized. The 75 subjects were randomized into five treatment groups and euthanized on days 3, 7, and 15 post-wounding. All animals underwent a one-week acclimatization period prior to experimental procedures.
2. **Wound induction:** Incisional wounds were created on the dorsal region following administration of ketamine (75-100 mg/kg body weight) and xylazine (5-10 mg/kg body weight) anesthesia, resulting in doses of 15-25 mg ketamine and 1-2.5 mg xylazine per animal. Incisions were made using a sterile scalpel after confirming adequate anesthesia (Krissanti et al., 2023).
3. **Intervention protocol:** Treatment was administered according to group allocation: negative control

(healthy, unwounded), positive control (povidone iodine), and experimental groups receiving Clitoria ternatea L. extract cream at concentrations of 5%, 10%, or 15% (Rosmarwati, Ellistasari and Kusumawardani, 2023).

D. Research Instrumens

Wound tissue specimens were harvested for histological and immunohistochemical analyses as follows:

1. Optimization of immunohistochemical staining for IL-6 and EGF detection.
2. Hematoxylin-Eosin (HE) staining for assessment of cutaneous re-epithelialization.
3. Immunohistochemical staining for quantification of IL-6 and EGF expression.

Protein expression was evaluated based on staining intensity and percentage of positive cells using microscopy at 400× magnification. All assessments were validated by a specialist in anatomical pathology at the research facility.

E. Statistical Analysis

Data were analyzed using the Kruskal-Wallis test to evaluate IL-6 and EGF expression, followed by Mann-Whitney post-hoc tests due to non-normal distribution and heterogeneity of variance. Re-epithelialization was similarly analyzed using Kruskal-Wallis non-parametric methodology with subsequent Mann-Whitney comparisons. The relationship between IL-6 and EGF expression was assessed using Spearman's correlation coefficient. All statistical analyses were performed using *SPSS 26.0* for Windows with significance established at $p<0.05$ (95% confidence level).

RESULT AND DISCUSSION

A. IL-6 Expression Analysis

Clitoria ternatea L. extract demonstrated a significant effect on enhancing the expression of IL-6 and EGF, contributing to accelerated wound healing (Biosciences, 2000).

The study evaluated various concentrations (5%, 10%, 15%) of Clitoria ternatea L. extract in comparison with a negative control group (healthy) and a positive control group (betadine). Based on the expression category analysis, the results revealed the following patterns: The healthy control group exhibited 0% IL-6 expression throughout the study period. In contrast, the betadine group showed strong expression (66.67%) on days 3 and 7, with a notable decrease by day 15.

On day 3, all Clitoria ternatea L. treatment groups (5%, 10%, and 15%) demonstrated 100% strong IL-6 expression, indicating a robust initial inflammatory response that facilitates tissue regeneration. By day 7, the 5% and 10% concentration groups maintained strong expression, while the 15% concentration group showed reduced expression (33.3% weak expression), suggesting a transition from the inflammatory phase to the proliferative phase. By day 15, IL-6 expression had significantly decreased in the 5% and 10% concentration groups, whereas the 15% concentration group maintained strong expression. This pattern indicates a progressive transition to the remodeling phase across all treatment groups (Johnson et al., 2020; Nabila Latifa Hafizsha et al., 2021; Putri, Fakih and Suarantika, 2024).

The study examined IL-6 expression in incision wounds of Sprague Dawley rats at three time points: days 3, 7, and 15. The findings indicated that administration of Clitoria ternatea L. extract at concentrations of 5%, 10%, and 15% resulted in comparable or superior IL-6 expression compared to control groups on day 3. IL-6 functions as a primary inflammatory mediator that activates the local immune response to tissue damage. The significant elevation in IL-6 expression observed on day 3 reflects its role in

recruiting macrophages and neutrophils, which are essential for removing necrotic tissue and initiating the proliferation phase (Widiyanto, Yuniarifa and Purnamasari, 2023) .

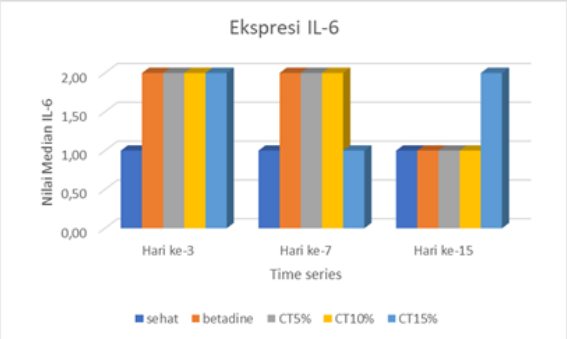


Figure 1. IL-6 Expression Levels Across Different Concentrations of Clitoria ternatea L. Extract.

Regarding IL-6 expression patterns: On day 3, IL-6 expression was elevated in the treatment group receiving 10% Clitoria ternatea flower extract compared to the negative control group. A progressive decrease in IL-6 expression was observed on days 7 and 15, indicating the transition from the inflammatory phase to the proliferative phase. IL-6 plays a crucial role during the early inflammatory stage of wound healing by attracting macrophages and neutrophils to the wound site, subsequently stimulating collagen production by fibroblasts. A marked increase in IL-6 expression was particularly evident in the groups treated with 10% and 15% Clitoria ternatea L. extract, suggesting that these concentrations effectively accelerate the initial inflammatory response (Lina Noviyanti Sutardi et al., 2022).

Table 1. Statistical Analysis of IL-6 Expression Based on Concentration Group of *Clitoria ternatea* L. Extract.

No.	Variables	Termination time (days)	n	Treatment group	Median (minimum - maximum)	*p-value*
1	IL-6 Expression	3	3	healthy	1(1-1)	0.39
			3	betadine	2(1-2)	
			3	*C. ternatea*5%	2(2-2)	
			3	*C. ternatea*10%	2(2-2)	
			3	*C. ternatea*15%	2(2-2)	
2		7	3	healthy	1(1-1)	0.21
			3	betadine	2(1-2)	
			3	*C. ternatea* 5%	2(2-2)	
			3	*C. ternatea* 10%	2(2-2)	
			3	*C. ternatea*15%	1(1-2)	
3		15	3	Healthy	1(1-1)	0.21
			3	betadine	1(1-2)	
			3	*C. ternatea*5%	1(1-1)	
			3	*C. ternatea*10%	1(1-1)	
			3	*C. ternatea*15%	2(1-2)	

Statistical analysis using the Kruskal-Wallis test revealed no significant differences in IL-6 expression, EGF levels, and re-epithelialization between treatment groups. Due to non-normal distribution and heterogeneity of the data, post hoc analysis was conducted using the Mann-Whitney test to compare the Clitoria

ternatea L. extract cream treatment groups with the betadine control group across all observation periods.

Analysis of IL-6 expression yielded the following significance values (p-values): day 3: p = 0.39; day 7: p = 0.21; day 15: p = 0.21. These results indicate that despite observable variations in IL-6 expression between experimental groups, the differences did not reach statistical significance.

Table 2. IL-6 Category Score Expression

No.	N	Group	Day 3 Expression		Day 7 Expression		Day 15 Expression	
			Weak	Strong	Weak	Strong	Weak	Strong
1	3	Healthy	3	0	3	0	3	0
2	3	betadine	1	2	1	2	2	1
3	3	*C. ternatea* 5%	0	3	0	3	3	0
4	3	*C. ternatea* 10%	0	3	0	3	3	0
5	3	*C. ternatea* 15%	0	3	2	1	1	2

Score 1: weak IL-6 expression
Score 2: strong IL-6 expression

Histopathological assessment combined with statistical analysis (Kruskal-Wallis and Mann-Whitney post hoc tests) demonstrated that various concentrations of Clitoria ternatea L. extract produced effects that were statistically comparable to the betadine control group. However, examination of median IL-6 expression values suggested favorable outcomes with Clitoria ternatea L. extract treatment (Johnson et al., 2020).

In summary, Clitoria ternatea L. extract enhances IL-6 expression during the initial wound healing phase, thereby accelerating the inflammatory response and promoting collagen synthesis essential for tissue regeneration. Notably, IL-6 expression diminished by day 15, particularly in the 5% and 10% concentration groups, signifying resolution of inflammation and facilitating more rapid wound healing without the complications associated with prolonged inflammatory processes (Kalangi, 2014; Cucci et al. , 2021).

B. EGF Expression Analysis

Epidermal Growth Factor (EGF) is a critical mediator that stimulates epithelial cell proliferation, an essential component of wound re-epithelialization (Wulandari and Oktariana, 2024). Immunohistochemical analysis demonstrated EGF expression localized to cell membranes, with notably enhanced staining intensity observed in specimens treated with Clitoria ternatea L. extract.

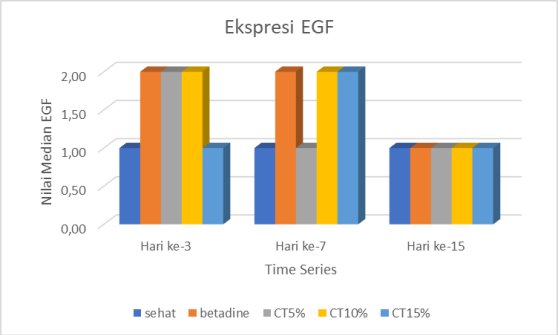


Figure 2. EGF Expression Levels Across Different Concentrations of Clitoria ternatea L. Extract

The control group (healthy) demonstrated no EGF expression (0%), while the positive control group (betadine) exhibited strong EGF expression (66.67%) on days 3 and 7, followed by a complete reduction to 0% by day 15. Analysis of the Clitoria ternatea L. treatment groups revealed distinct temporal patterns of EGF expression. On day 3, both the 5% and 10% concentration groups demonstrated robust EGF expression (100% strong), whereas the 15% concentration group showed reduced expression (33.3% weak). By day 7,

differential responses were observed: the 5% concentration group exhibited weak expression (33.3%), the 10% concentration group maintained strong expression (100%), and the 15% concentration group showed moderately strong expression (66.7%). These changes support the transition from inflammatory to proliferative phases of wound healing. By day 15, both the 5% and 10% concentration groups showed complete reduction in EGF expression (0%), while the 15% concentration group maintained minimal expression (33.3% weak), demonstrating effective progression toward tissue regeneration and remodeling.

Notably, enhanced EGF expression was observed on days 7 and 15 specifically in the 10% and 15% Clitoria ternatea L. extract treatment groups, suggesting significant potential for accelerating tissue regeneration processes.

Comparative analysis revealed that on day 3, EGF expression was elevated in the 5% and 10% extract groups, with a slightly higher but statistically non-significant increase in the 15% group. By day 7, the 10% and 15% concentration groups demonstrated significantly increased EGF expression, while the 5% group showed no significant difference from controls. This pattern aligns with EGF's established role in stimulating cell proliferation and epithelial differentiation. The subsequent decrease in EGF expression observed across all treatment groups by day 15 indicates appropriate progression to the remodeling phase of wound healing (Tottoli et al., 2020; Malaha et al., 2023; Wulandari and Oktariana, 2024).

Table 3. Analysis of EGF Expression Based on Different Concentrations of *Clitoria ternatea* L. Extract

No.	Termination time (days)	n	Treatment group	Median (minimum-maximum)	*p*-value
1	3	3	healthy	1(1-1)	0.21
		3	betadine	2(1-2)	
		3	*C. ternatea*5%	2(2-2)	
		3	*C. ternatea*10%	2(2-2)	
		3	*C. ternatea*15%	1(1-2)	
2	7	3	healthy	1(1-1)	0.43
		3	betadine	2(1-2)	
		3	*C. ternatea*5%	1(1-2)	
		3	*C. ternatea*10%	2(2-2)	
		3	*C. ternatea*15%	2(1-2)	
3	15	3	healthy	1(1-1)	0.39
		3	betadine	1(1-1)	
		3	*C. ternatea*5%	1(1-1)	
		3	*C. ternatea*10%	1(1-1)	
		3	*C. ternatea*15%	1(1-2)	

Statistical analysis of EGF expression using the Kruskal-Wallis test revealed no significant differences among treatment groups across all time points (day 3: $p = 0.21$; day 7: $p = 0.43$; day 15: $p = 0.39$). These findings indicate that EGF expression in Clitoria ternatea L. extract treatment groups was comparable to the betadine control group.

Temporal analysis demonstrated that EGF expression initiated on day 3, with particularly notable increases in the 10%

Clitoria ternatea L. extract treatment group. This expression progressively increased through day 7 and reached maximum levels by day 15, suggesting effective stimulation of epithelial tissue regeneration. These results confirm that Clitoria ternatea L. extract at various concentrations elicited EGF expression patterns similar to betadine, with no statistically significant variations observed among treatment groups (Wulandari and Oktariana, 2024) .

Table 4. EGF Expression score categories

No	n	Group	EGF Expression Category Day 3		EGF Expression Category Day 7		EGF Expression Category Day 15	
			Weak	Strong	Weak	Strong	Weak	Strong
1	3	healthy	3	0	3	0	3	0
2	3	betadine	1	2	1	2	3	0
3	3	*C. ternatea* 5%	0	3	2	1	3	0
4	3	*C. ternatea* 10%	0	3	0	3	3	0
5	3	*C. ternatea* 15%	2	1	1	2	2	1

These findings suggest that Clitoria ternatea L. possesses therapeutic potential in facilitating the early proliferative phase of wound healing, though its efficacy was not significantly different from betadine within the experimental timeframe. Additional research is warranted to elucidate the specific mechanisms through which this extract influences wound healing pathways, with particular emphasis on EGF-mediated processes.

C. Re- Epithelialization Result

Re-epithelialization, the terminal phase of wound healing, is characterized by regeneration of the epidermal layer to establish complete coverage of the wound surface.

1. Day 3: All experimental groups exhibited minimal epithelial thickness, consistent with the early inflammatory phase of wound healing.
2. Day 7: The 10% Clitoria ternatea L. extract treatment group demonstrated enhanced epithelial thickness compared to the negative control, indicating accelerated progression into the proliferative phase.
3. Day 15: Optimal re-epithelialization was observed in the 5% and 10% Clitoria ternatea L. extract treatment groups, with efficacy comparable to the povidone-iodine (betadine) positive control.

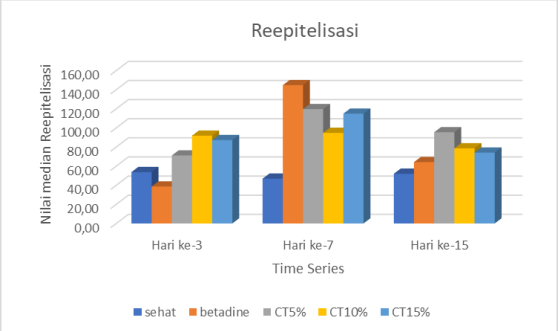


Figure 3. Re-epithelialization Measurements Following Application of Clitoria ternatea L. Extract

The notable enhancement in EGF expression observed across treatment groups suggests that Clitoria ternatea L. extract facilitates accelerated re-epithelialization, with optimal effects at concentrations of 10% and 15% (Sumartini and Ikrawan, 2020; Lina Noviyanti Sutardi et al., 2022).

Nevertheless, statistical analyses indicated that the differences in epithelial thickness measurements between experimental groups did not reach

statistical significance at any of the designated observation timepoints.

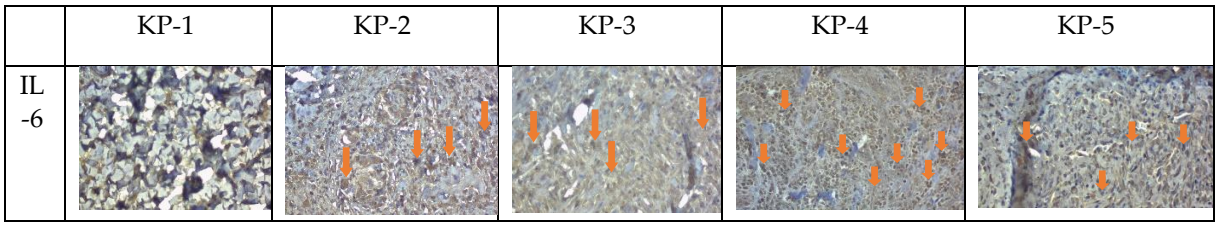


Figure 4. Histopathologic Analysis of Interleukin-6:
Skin Histopathology Analysis: IL-6 expression colored brown; (KP-1) skin histopathology of negative/healthy control group, (KP-2) skin histopathology of positive control group with betadine application, (KP-3) skin histopathology of group with 5% *Clitoria ternatea* L extract cream, (KP-4) skin histopathology of group with 10% *Clitoria ternatea* L extract cream, (KP-5) skin histopathology of group with 15% *Clitoria ternatea* L extract cream

Table 5. Quantitative Assessment of Re-epithelialization Following Application of Clitoria ternatea L. Extract.

Analysis of Re-epithelialization Data

No	Termination time (days)	n	Treatment group	Mean±sSD	*p*-value
1	3	3	healthy	54.34±3.73	0
		3	betadine	36.23±5.13	
		3	C. ternatea5%	77.92±17.20	
		3	C. ternatea10%	77.02±35.51	
		3	C. ternatea15%	88.07±15.10	
2	7	3	healthy	46.98±3.37	0
		3	betadine	151.37±16.72	
		3	C. ternatea5%	138.02±36.06	
		3	C. ternatea10%	102.11±21.48	
		3	C. ternatea15%	111.04±12.29	
3	15	3	healthy	54.99±6.85	0
		3	betadine	66.31±4.46	
		3	C. ternatea5%	96.04±7.97	
		3	C. ternatea10%	82.92±23.31	
		3	C. ternatea15%	74.46±12.29	

IL-6, a proinflammatory cytokine, plays a crucial role in wound healing by facilitating immune cell recruitment to the injury site. Quantitative analysis at day 3 demonstrated significantly elevated IL-6 expression across all Clitoria ternatea L. extract treatment groups (5%, 10%, and 15%), indicating an appropriate initial inflammatory response essential for wound healing cascade initiation. The betadine control group exhibited comparable IL-6 expression patterns, suggesting similar inflammatory mechanism activation as observed with C. ternatea L. treatment. By day 15, IL-6 expression had markedly decreased across all experimental groups, signifying resolution of the inflammatory phase and progression to the tissue remodeling phase.

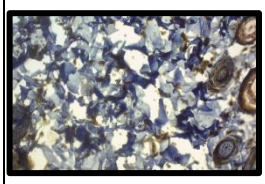
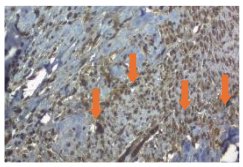
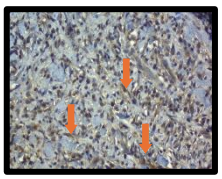
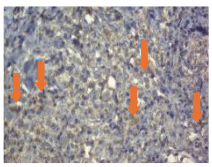
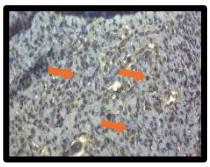
	KP-1	KP-2	KP-3	KP-4	KP-5
EGF					

Figure 5. Epidermal Growth Factor (EGF)

Histopathology analysis of the skin: (KP-1) skin histopathology of the negative control group, (KP-2) skin histopathology of the positive control group with the use of betadine, (KP-3) skin histopathology of the group using 5% *Clitoria ternatea* L extract cream, (KP-4) skin histopathology of the group using 10% *Clitoria ternatea* L extract cream, (KP-5) skin histopathology of the group using 15% *Clitoria ternatea* L extract cream.

Epidermal Growth Factor (EGF) serves as a critical mediator in wound healing by promoting proliferation of epithelial cells and fibroblasts. Temporal analysis revealed elevated EGF expression in Clitoria ternatea L. extract-treated groups on days 3 and 7, with expression patterns comparable to the betadine control group. This finding suggests that C. ternatea L. extract effectively stimulates epithelial proliferation, thereby facilitating wound re-epithelialization. By day 15, a notable decrease in EGF expression was observed across all experimental groups, indicating the conclusion of the proliferative phase and transition to the remodeling phase of wound healing.

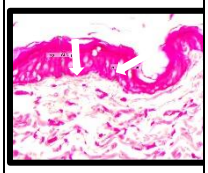
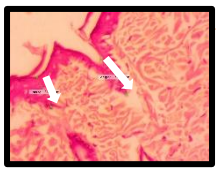
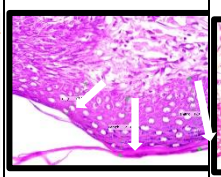
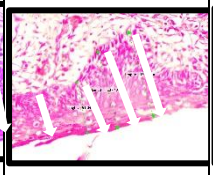
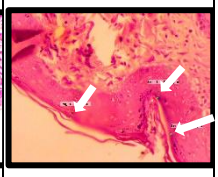
	KP-1	**KP-2**	**KP-3**	**KP-4**	**KP-5**
Re-epithelialization					

Figure 5. Skin Histopathology Analysis:

(KP-1) skin histopathology of the negative control group, (KP-2) skin histopathology of the positive control group with betadine, (KP-3) skin histopathology of the group using 5% *Clitoria ternatea* L extract cream, (KP-4) skin histopathology of the group using 10% *Clitoria ternatea* L extract cream, (KP-5) skin histopathology of the group using 15% *Clitoria ternatea* L extract cream.

Re-epithelialization was assessed using Hematoxylin and Eosin (H&E) staining to evaluate epithelial thickness in the wound area. **Day 3**: All treatment groups demonstrated increased re-epithelialization, with the CT 15% group exhibiting the highest degree of epithelial regeneration, followed by CT 5% and CT 10% groups, all of which displayed greater epithelial thickness compared to the betadine control. **Day 7**: Maximum epithelial thickness was observed in the betadine group, with the CT 5% group showing the second highest measurement. **Day 15**: The CT 5% group exhibited the most pronounced epithelial thickness, while the betadine group demonstrated comparatively reduced epithelial thickness.

Previous investigations into the wound healing properties of Clitoria ternatea L. extract have been conducted

with varying methodological approaches. One notable study evaluated the efficacy of telang flower extract cream on Vascular Endothelial Growth Factor (VEGF) expression and angiogenesis in incisional wounds of male Sprague Dawley rats. The findings demonstrated. That Clitoria ternatea extract cream was well-tolerated and significantly enhanced VEGF expression and angiogenesis. These results suggest that this formulation represents a safe, cost-effective alternative for wound management protocols (Irwandi, 2024).

CONCLUSION

Collectively, these findings indicate that Clitoria ternatea L. extract at various concentrations demonstrates significant potential for enhancing wound re-epithelialization, particularly during the initial phases of wound healing.

Histopathological evaluation revealed that Clitoria ternatea L. extract at different concentrations produced effects comparable to betadine in upregulating IL-6 expression, EGF levels, and re-epithelialization during incisional wound healing processes. Notably, the 5% and 15% concentrations exhibited superior efficacy in promoting epithelial thickness during both proliferation and remodeling phases. These data suggest that Clitoria ternatea L. extract represents a promising alternative therapeutic agent for the management of incisional wounds.

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