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In Vivo Analysis of *Pandanus tectorius* Fruit Extract Gel: Modulation of Epithelialization, Collagen Synthesis, and Fibroblast Proliferation in Wound Healing

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ABSTRACT

Herbal therapies are increasingly explored for wound management due to their perceived safety compared with synthetic agents. Pandanus tectorius contains bioactive compounds with potential benefits for tissue repair; however, evidence supporting its efficacy in incision wound healing remains limited. This study evaluated the effects of Pandanus tectorius ethanol extract gel on epithelialization, collagen density, and fibroblast proliferation in incision wounds of Rattus norvegicus. A post-test-only control-group design used 30 white rats allocated into five groups: positive control (Bioplacenton®), negative control (gel base), and extract gel at 10%, 20%, and 30%. Wounds were treated twice daily for 10 days, followed by histological evaluation of epithelialization, fibroblast count, and collagen density. Data were analyzed using ANOVA with Duncan's post hoc test. Fibroblast proliferation differed significantly among groups ($p < 0.001$; $\eta^2 = 0.590$), with the 20% and 30% extract groups showing the highest counts. Collagen density exhibited non-normal distribution in the negative control but homogeneous variances overall. ANOVA revealed no significant differences in collagen density; however, Duncan's test indicated a trend toward increased formation in the 30% group. The results demonstrate that Pandanus tectorius extract gel enhances tissue regeneration by promoting epithelialization and fibroblast proliferation. Despite non-significant collagen improvements, the upward trend supports its role in extracellular matrix formation. Overall, Pandanus tectorius fruit extract gel shows promise as a natural wound-healing agent, warranting further research on optimal dosing and clinical applicability.

INTRODUCTION

A wound is damage to a portion of the cellular and tissue structure, disrupting the skin's anatomical function (Bernatchez & Bichel, 2023; Sullivan & Myers, 2022; Winston et al., 2023). Damage to the skin's anatomical structure and function triggers the body's response, initiating the wound healing process. The wound healing process consists of three stages: the first is the inflammatory phase, the second is the proliferative phase, and the third is the maturation phase. Wound healing will fail if inhibiting factors exist, including infection, hematoma, and foreign objects. Wound treatment aims to reduce risk factors that inhibit wound healing, accelerate the healing process, and minimize the incidence of infected wounds (Aminuddin et al., 2020).

Medicinal plants exhibit a wide range of biological and medicinal activities; they are generally safe, readily available, and inexpensive. One medicinal plant commonly used as a traditional remedy for incision wound healing is the fruit of *Pandanus tectorius*. *Pandanus tectorius* contains several bioactive compounds, including phenolics, flavonoids, steroids, triterpenoids, saponins, and glycosides. These bioactive compounds are significant due to their advantages as anti-inflammatory agents, antioxidants, and antimicrobial agents, while also facilitating collagen formation, cell growth, and blood vessel formation (Cedillo-Cortezano et al., 2024; Ningsih et al., 2023).

Flavonoids act by three mechanisms to prevent free radical formation: the first is to slow the formation of reactive oxygen species (ROS), the second is to destroy ROS, and the third is to control or protect against them with antioxidants (Ningsih et al., 2023). Flavonoids can enhance macrophage stimulation, which can lead to increased production of transforming growth factor (TGF). TGF is also responsible for increasing fibroblasts, improving the extracellular matrix (ECM), and increasing collagenase in the wound healing process (Amfotis, Suarna, et al., 2022).

Despite the traditional use of *Pandanus tectorius* in wound care, microscopic evidence of its effects on the cellular mechanisms of wound healing remains scarce. Most previous studies have focused on macroscopic wound closure or antimicrobial activity, leaving a knowledge gap regarding the histological impact on *epithelialization*, fibroblast proliferation, and collagen synthesis—key cellular processes that determine healing quality and tissue strength. This gap underscores the urgency of conducting histopathological investigations to validate the therapeutic claims of *Pandanus tectorius* and to establish a scientific basis for its clinical application.

Based on the above description, this study aims to further investigate the benefits of an ethanol extract of *Pandanus tectorius* fruit as a wound-healing agent. The novelty of this study lies in the comprehensive microscopic observation of wound healing at the cellular level, specifically evaluating three critical histological parameters—*epithelialization*, collagen density, and fibroblast proliferation—simultaneously in an incision wound model. The aim is to determine the effect of the ethanol extract of *Pandanus tectorius* fruit in three different concentrations (10%, 20%, and 30%) on incision wound healing by measuring *epithelialization*, collagen density, and fibroblast activity.

METHOD

This study used a posttest control group design. The samples were divided into five groups: the positive control, the negative control, and the Treatment Groups with 10%, 20%, and 30% *Pandanus tectorius* fruit ethanol extract gel, each consisting of six white rats. The skin tissue specimens were then stained with H&E (Hematoxylin-Eosin) to assess the number of epithelial and fibroblast cells, and with Masson's Trichrome to evaluate collagen formation. Histopathological slides were observed under a microscope at 400x magnification in four fields of view, and microscopic changes were recorded based on the variables examined. This research procedure complies with the code of ethics issued by the ethics committee (Approval No. I/060/UHT.KEPK.03/VIII/2025).

Before treatment, the rats were acclimatized for 7 days. During acclimatization, the rats were provided with food and water ad libitum. The cages and drinking bowls were cleaned

regularly. A total of 30 rats were anesthetized with 10 mg/kg ketamine and 20 mg/kg xylazine, administered intramuscularly. The backs of the rats were shaved to a smooth area of 3 cm x 2 cm, then cleaned with 96% alcohol. Marks were made at 2 cm intervals using a sterile scalpel, 2 cm long (Amfotis, Made, et al., 2022).

The wounds were cleaned with alcohol. After 10 minutes the wound was treated, namely Control (-) was given a gel base of 1 gram, Control (+) was given a gel base of 1 gram, Treatment Group 1 was given a gel extract of *Pandanus tectorius* fruit with a concentration of 10% as much as 1 gram, Treatment Group 2 was given a gel extract of *Pandanus tectorius* fruit with a concentration of 20% as much as 1 gram, and Treatment Group 3 was given a gel extract of *Pandanus tectorius* fruit with a concentration of 30% as much as 1 gram. Treatment was administered twice a day, at 07:00 in the morning and 14:00 in the afternoon. Skin histology slides were prepared using paraffin and hematoxylin-eosin staining method to determine the number of epithelial cells, fibroblasts, and Masson's trichomes to see the collagen formation. The average number of epithelial cells and fibroblasts was calculated by observing an even distribution under a microscope and then photographing them at 100x magnification.

Using Image Raster, a 50 μm x 50 μm box was created, and the number of fibroblasts and epithelial cells within the box was manually counted. Collagen was quantified using Adobe Photoshop CS3 version 9.0 software and Image Raster at 400x magnification. The amount of blue collagen was calculated as a percentage of the collagen pixel area compared to the total tissue pixel area (Amfotis, Suarni, et al., 2022; Dwita et al., 2020; Gunawan et al., 2019; Luthfi et al., 2020). % = (collagen pixel area)/(Pixel area throughout the collagen) x 100%.

RESULT AND DISCUSSION

Table 1. Test Result of Spesific Parameters for Ethanol Extract of *Pandanus tectorius* Fruit

Specific Parameter		Result
Identity	Extract Name	*Pandanus tectorius* Extractum
	Species of the plant	*Pandanus tectorius*
	Plant part used	Fruit
	Family	Pandanaceae

Specific parameter testing is carried out to guarantee that the raw materials used for making the extract are genuine, traceable, and of proper quality. From the identification process, the material was confirmed to be *Pandanus tectorius* extractum, which matches the botanical characteristics of the *Pandanus tectorius* species from the Pandanaceae family. The plant part used was the fruit, in accordance with the literature, which states that this part is the primary source of secondary metabolites, such as flavonoids, terpenoids, and alkaloids, which play roles in biological activities, including wound healing (Setiani et al., 2025).

Table 2. Result of Phytochemical Screening for Ethanol Extract of *Pandanus tectorius* fruit

Secondary Metabolite	Result
Flavonoid (mg/mL)	169,48

Alkaloid (mg/mL)	12,24
Saponin (mg/mL)	0,053
Terpenoid (mg/mL)	12,76

Phytochemical screening results showed that the ethanol extract of *Pandanus tectorius* fruit was dominated by flavonoids (169.48 mg/mL), followed by terpenoids (12.76 mg/mL) and alkaloids (12.24 mg/mL), while saponins were found in very low amounts (0.053 mg/mL). This flavonoid dominance indicates that the extract's primary biological activity is primarily antioxidant and anti-inflammatory (Husna et al., 2022). Flavonoids are recognized for their ability to neutralize free radicals, suppress inflammatory mediators like TNF-α and COX-2, and stimulate fibroblast growth as well as collagen formation. These actions make them highly important in the wound-healing process (Zulkefli et al., 2023).

The presence of terpenoids and alkaloids at moderate levels also supports the extract's antimicrobial and anti-inflammatory activities (Sanjaya et al., 2023). Meanwhile, the low saponin content indicates a minimal risk of irritation in topical formulations. Overall, this secondary metabolite profile is consistent with in vivo findings that the extract gel is able to increase epithelial cell count and fibroblast proliferation.

Table 3. Descriptive Analysis Results of Histopathological Parameters

Group	The Average of Epithelial Cells	The Average of Fibroblast Cells	MT (%)
Control (-)	16.22 ± 3.78	9.22 ± 4.67	15.00 ± 10.00
Control (+)	17.56 ± 3.08	12.89 ± 4.87	26.67 ± 18.07
TG1	20.61 ± 3.90	15.00 ± 5.42	22.50 ± 13.32
TG2	29.28 ± 5.21	24.06 ± 3.25	30.00 ± 10.00
TG3	25.22 ± 6.44	25.28 ± 9.01	35.00 ± 7.07

Based on Table 3, Treatment Group 2 with the administration of *Pandanus tectorius* fruit ethanol extract gel with a concentration of 20% showed the highest average number of epithelial cells (29.28 ± 5.21), followed by Treatment Group 3 with the administration of *Pandanus tectorius* fruit ethanol extract gel with a concentration of 30% (25.22 ± 6.44). For the results on the number of fibroblast cells, Treatment Group 3 showed the highest average (25.28 ± 9.01), followed by Treatment Group 2 with results (24.06 ± 3.25). The highest percentage of collagen density parameters was found in Treatment Group 3, with the administration of *Pandanus tectorius* fruit ethanol extract gel with a concentration of 30%, with results (35.00 ± 7.07%).

Table 4. Statistical Test Results for Histopathological Parameters (One-Way ANOVA)

Variable	F	df	p-value	Eta-squared (η^2)	Interpretation
The Average of Epithelial Cells	8.234	4, 25	<0.001**	0.568	Significant
The Average of Fibroblast Cells	8.979	4, 25	<0.001**	0.590	Significant
MT (%)	2.293	4, 25	0.088	0.268	Not Significant

**p<0.001; df between groups = 4; df within groups = 25

ANOVA results showed highly significant differences in the number of epithelial cells ($F = 8.234$; $p < 0.001$) and fibroblasts ($F = 8.979$;$p < 0.001$) between the Treatment Groups. A

large effect size was indicated by an eta-squared value greater than 0.50 for both variables, indicating that more than 50% of the variability could be explained by the treatment differences. Conversely, there was no significant difference in the percentage of MT between groups (F = 2.293; p = 0.088).

Number of Epithelial Cells

Table 5. Duncan's Test Results for Mean Epithelial

Group	N	Subset 1	Subset 2	Subset 3
Control (-)	6	16.22[a]	-	-
Control (+)	6	17.56[a]	-	-
TG1	6	20.61[ab]	20.61[ab]	-
TG3	6	-	25.22[bc]	25.22[bc]
TG2	6	-	-	29.28[c]
Sig.		0.133	0.098	0.143

Groups with different letters indicate significant differences (α=0.05)

The results showed that the number of epithelial cells differed between Treatment Groups, with Treatment Group 2 showing the highest increase and significantly different from the control groups (C- and C+). This indicates that treatment in Group 2 was more effective in stimulating epithelial cell proliferation. High epithelial cell proliferation indicates improved tissue regeneration, a crucial factor in the healing and repair process. Treatment Groups 1 and 3 were intermediate, meaning the treatment effect in these two groups was not as strong as that of Treatment Group 2, but still showed an increase compared to the control. Meanwhile, the C- and C+ groups did not show a significant difference, indicating that the control treatment did not significantly increase the number of epithelial cells.

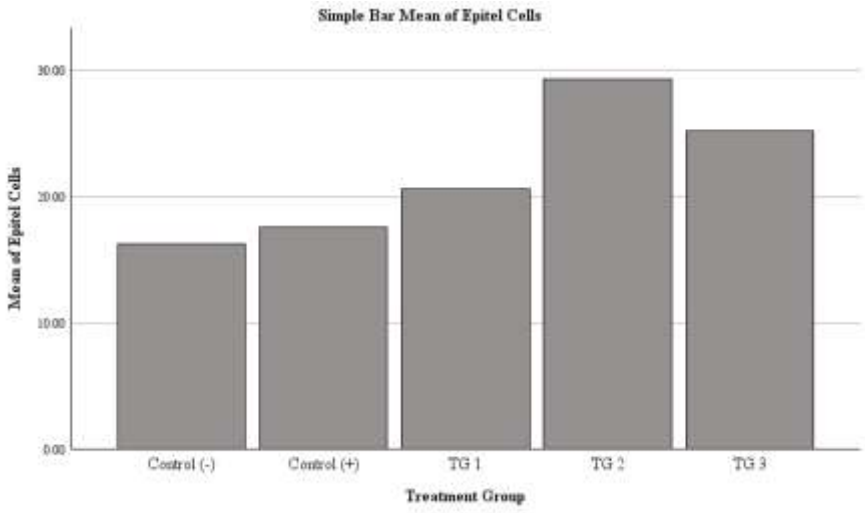

Figure 1. Simple Bar Mean of Epitel Cells

Overall, these results demonstrate that Treatment Group 2 has the greatest potential to increase epithelial cells proliferative activity, likely through growth factor stimulation or increased collagen synthesis, which supports new tissue formation. This finding aligns with the

principle that increased epithelial cell activity plays a crucial role in accelerating wound healing and tissue regeneration (Zulkefli et al., 2023).

Total of the Fibroblast Cells

Table 6. Duncan's Test Results: Mean of The Fibroblast Cell Count

Group	N	Subset 1	Subset 2
Control (-)	6	9.22[a]	-
Control (+)	6	12.89[a]	-
TG1	6	15.00[a]	-
TG2	6	-	24.06[b]
TG3	6	-	25.28[b]
Sig.		0.113	0.717

Groups with different letters indicate significant differences (α=0.05)

Duncan's test results indicate that the number of fibroblast cells can be grouped into two homogeneous subsets that are significantly different from each other. The first subset (notation a) comprises the C-, C+, and Treatment Group 1 groups, which did not show significant differences from one another (p = 0.113). The second subset (notation b) includes Treatment Groups 2 and 3, which also did not differ significantly from each other (p = 0.717), but were significantly different from the first subset, this indicates that treatment using the *Pandanus tectorius* fruit extract gel at concentrations of 20% and 30% significantly increased the number of fibroblast cells compared to the negative control , positive control, and Treatment Group 1 (TG1). This increase in fibroblast numbers indicates that both concentrations play an active role in accelerating the process of new connective tissue formation during incision wound healing.

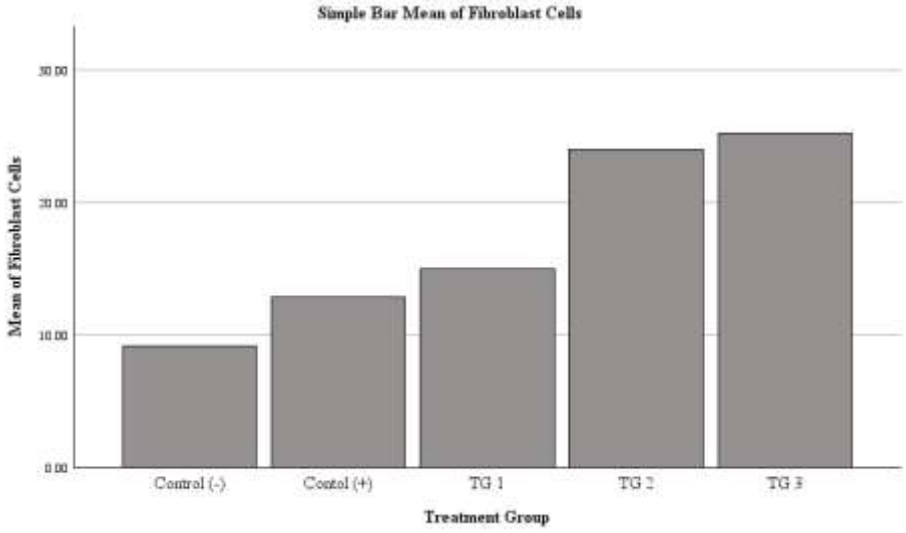

Figure 2. Simple Bar Mean of Fibroblast Cells

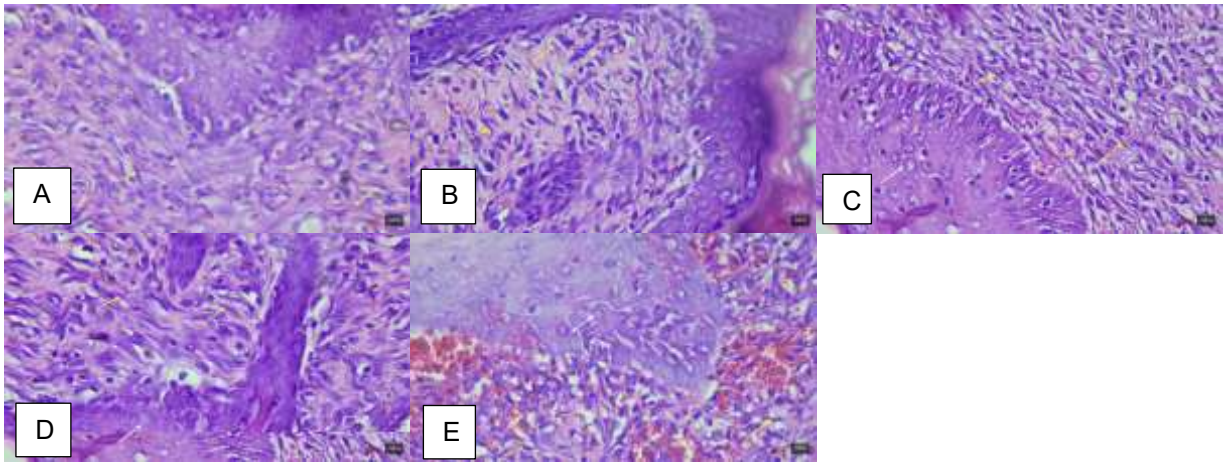

Figure 3. (A) Control positive, (B) Control negative, (C) Treatment Group 1, (D) Treatment Group 2, (E) Treatment Group 3, white arrows indicate epithelial cells, yellow arrows indicate fibroblast cells

Collagen Density

Table 7. Duncan Test Results for Collagen Density (MT %)

Group	N	Subset 1	Subset 2
C-	6	15.00[a]	-
TG1	6	22.50[ab]	22.50[ab]
C+	6	26.67[ab]	26.67[ab]
TG2	6	30.00[ab]	30.00[ab]
TG3	6	-	35.00[b]
Sig.		0.062	0.118

Groups with different letters indicate significant differences (α=0.05)

The ANOVA analysis results showed that the difference in collagen density values between groups was not statistically significant (p = 0.088). However, Duncan's further test results revealed a trend of differences between Treatment Groups. The first subset (notation a) consisted of group C(-), Treatment Group 1, C(+), and Treatment Group 2, which showed similar collagen density values and were not significantly different. The second subset (notation b) included Treatment Groups 1, C(+), Treatment Group 2, and Treatment Group 3, which also showed similar values within the groups. Some groups, such as Treatment Group 1, C(+), and Treatment Group 2, had the notation ab, indicating an intermediate position and not significantly different from the other two subsets. Overall, only Treatment Group 3 showed a trend of having higher collagen density values than the control group C(-), but this difference did not reach statistical significance in the ANOVA analysis. These findings indicate a trend of increasing MT values at higher concentrations of *Pandanus tectorius* fruit extract (30%), although the increase is not substantial enough to be considered statistically significant.

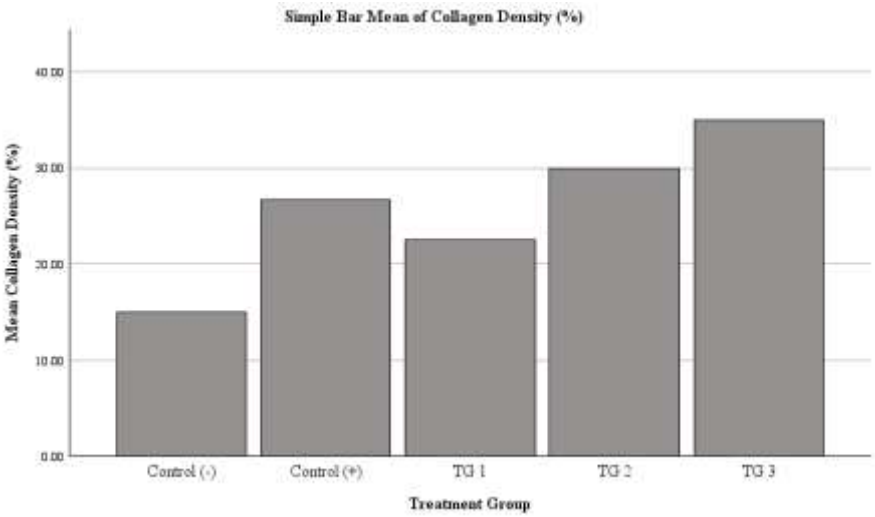

Figure 4. Simple Bar Mean of Collagen Density (%)

There was no statistically significant difference in the percentage of MT ($p = 0.088$). Although there was an increasing trend from C- (15.00%) to TG3 (35.00%), and Duncan's test showed that TG3 tended to differ from C-, the high intersample variability prevented this difference from reaching statistical significance.

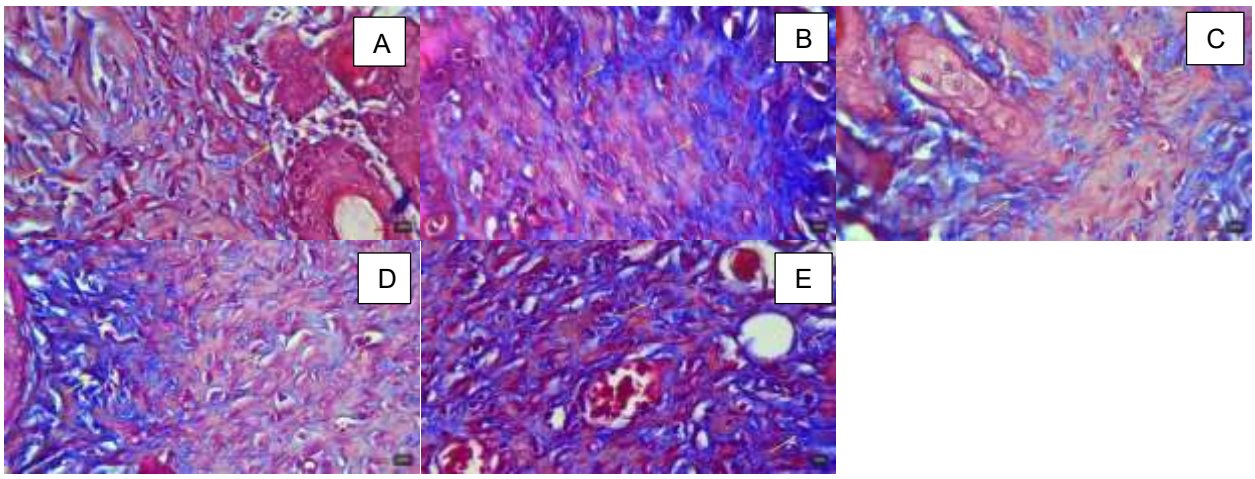

Figure 5. (A) Control positive, (B) Control negative, (C) Treatment Group 1, (D) Treatment Group 2, (E) Treatment Group 3, Collagen density

Duncan's test of epithelial counts showed significant differences between the Treatment Groups. Treatment Group 2 showed the highest results and was significantly different compared to the negative control (C–) and positive control (C+) groups. This indicates that administering *Pandanus tectorius* fruit ethanol extract gel at 20% concentration effectively stimulated epithelial cell proliferation compared with the other treatments. This effect stems from the content of active compounds in *Pandanus tectorius* fruit, such as flavonoids, tannins, alkaloids, and phenolics, which act as antioxidants, anti-inflammatories, and stimulants of tissue regeneration (Ningsih et al., 2023).Flavonoids have the ability to increase the expression of transforming growth factor-beta (TGF-β), which plays a role in epithelial cell proliferation

and differentiation (Amfotis, Suarni, et al., 2022). Treatment Groups 1 and 3 showed intermediate results, namely an increase compared to the control, but not as strong as Treatment Group 2. This indicates an optimal dose effect at a concentration of 20%, where higher doses are not always followed by a proportional increase in effect (dose-dependent plateau). Meanwhile, the control groups (C– and C+) showed no significant differences, indicating that without *Pandanus tectorius* extract treatment, the epithelial regeneration process was slower.

Duncan's test of the number of fibroblast cells revealed two homogeneous subsets with significant differences. The first subset, C(–), C(+), Treatment Group 1, had fewer fibroblasts than the second subset (Treatment Group 2 and Treatment Group 3). This indicates that administration of *Pandanus tectorius* fruit ethanol extract gel at 20% and 30% significantly increased the number of fibroblast cells compared with the control or the 10% extract concentration. The active compounds in *Pandanus tectorius* fruit, particularly flavonoids and phenolics, enhance fibroblast initiation and wound contraction. Furthermore, the antioxidant effects of flavonoids and phenolics also protect fibroblasts from oxidative stress-induced damage during the inflammatory phase(Ningsih et al., 2023). Treatment Group 3 showed the highest number of fibroblasts, indicating that increasing the extract concentration to 30% is still effective in stimulating fibroblast activity. However, the difference between Treatment Groups 2 and 3 was not statistically significant, indicating that the 20% extra concentration alone achieved the optimal biological effect without further dose increases.

The ANOVA analysis of collagen density differences between groups did not reach statistical significance ($p = 0.088$). However, a Duncan test was performed to determine a trend of increased collagen density in the group with the higher extract dose. Treatment Group 3 showed a trend toward higher collagen density compared to the other groups, although the difference was not statistically significant. Collagen is the main structural component of connective tissue, determining skin strength and elasticity. The increase in collagen density seen in the Treatment Group indicates that *Pandanus tectorius* extract plays a role in accelerating collagen deposition and maturation. The flavonoid content inhibits the synthesis of prostaglandins and other inflammatory mediators, including leukotrienes. Reducing the synthesis of prostaglandins and leukotrienes, which are inflammatory mediators, can accelerate the transition from inflammation to proliferation, thereby facilitating rapid wound healing. The saponins and phenols contained in *Pandanus tectorius* fruit extract can also increase monocyte growth, thereby influencing macrophage numbers. The increased presence of macrophages around wounds can increase the release of growth factors that facilitate proliferation, enhance fibroblast migration, and increase collagen production, all of which support rapid proliferation (Rifqi Efendi et al., 2023).

These effects suggest that *Pandanus tectorius* fruit has the potential to enhance the tissue remodeling phase after the inflammatory process has ended. Although the increase in collagen density was not statistically significant, the consistent upward trend in Treatment Group 3 indicates a significant biological effect. Nevertheless, these results demonstrate the therapeutic potential of *Pandanus tectorius* in supporting collagen formation and strengthening wound-healing tissue.

Although this study demonstrated the wound-healing potential of *Pandanus tectorius* ethanol extract gel, several limitations should be acknowledged. The collagen density parameter did not reach statistical significance, likely due to high variability between samples,

indicating that more consistent tissue sampling or additional microscopic fields may be needed in future research. This study also evaluated tissue only on day 10, providing a limited view of the healing process; therefore, observing multiple time points could offer a clearer picture of the progression of epithelialization, fibroblast activity, and collagen maturation. The use of a single wound model (incision wound) may also limit generalizability, suggesting that future studies should include other wound types. Additionally, the comparison was limited to Bioplacenton as positive control, and expanding comparison groups may help better assess the relative effectiveness of *Pandanus tectorius* extract.

CONCLUSION

This study demonstrates that ethanol extract gel from *Pandanus tectorius* fruit enhances wound healing by promoting epithelialization and fibroblast proliferation, aligning with its flavonoid-rich profile that provides antioxidant and anti-inflammatory benefits. While 20% and 30% concentrations showed superior responses, statistical overlap and high inter-animal variability prevent conclusive superiority claims for the 30% formulation; collagen density displayed only a non-significant upward trend, warranting further validation. Overall, the extract holds promise as a natural wound-healing agent. Future research should prioritize longitudinal studies with multiple time points (e.g., days 3, 7, 10, 14), larger sample sizes, molecular analyses of growth factors (TGF-β, VEGF, FGF) and inflammatory markers (TNF-α, IL-6), diverse wound models, dose optimization beyond 30%, and safety testing to elucidate mechanisms, reduce variability, and support clinical translation.

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