Bioeksperimen: Jurnal Penelitian Biologi
Volume 12, Issue 2, September 2026, pp. 226 – 238
p-ISSN: 2460-1365, e-ISSN: 2527-2799
https://journals2.ums.ac.id/bioeksperimen

Effect of topical collagen extract ointment from *Oreochromis niloticus* L. scales on incision wound healing in *Mus musculus* L.

Liliana Devita Sari[1)], Rudy Agung Nugroho*[1)], Retno Aryani[1)], Reni Kurniati[1)], Eko Kusumawati[1)]
[1]Departemen of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, 75117, Indonesia

*Corresponding e-mail: rudyagung.nugroho@fmipa.unmul.ac.id

How to cite:
Sari, L. D., Nugroho, R. A., Aryani, R., Kurniati, R., & Kusumawati, E. (2026). Effect of topical collagen extract ointment from Oreochromis niloticus L. scales on incision wound healing in Mus musculus L. Bioeksperimen: Jurnal Penelitian Biologi, 12(2), 226–238. https://doi.org/10.23917/bioeksperimen.v12i2.16418.

Article info

Article History:
Received: 23 May 2026, Revised: 13 July 2026, Available Online: 28 September 2026

Keywords:
Collagen, Ointment, Tilapia, Tilapia scales, Wound healing.

Abstract

Nile tilapia (*Oreochromis niloticus* L.) ranks among Indonesia's most heavily farmed freshwater fish, and the scales discarded during its processing are a rich, underused source of type I collagen, a structural protein of the extracellular matrix that supports skin repair and could serve as a natural ingredient in wound-care ointments. This study evaluated the physical properties of an ointment formulated with tilapia scale collagen extract and examined its effect on incision wound healing in mice (*Mus musculus* L.). Six groups were compared: K- (untreated), K+1 (bioplacenton), K+2 (ointment base only), and three collagen-ointment concentrations, P1 (5%), P2 (10%), and P3 (15%). Collagen was isolated by the acid-soluble collagen (ASC) method and characterized by Fourier-transform infrared (FTIR) spectroscopy; wound closure, tissue protein content (Lowry method), and hydroxyproline content were assessed over 14 days. All three ointment formulations met the general pharmaceutical standards for topical preparations. Wounds in every group closed gradually, reaching a mean closure of 87.4-98.2% by day 14 without a statistically significant difference among treatments, whereas protein and hydroxyproline assays showed that the 15% collagen ointment produced the lowest tissue protein (397.0 ± 8.55 µg/mL) and hydroxyproline (82.05 ± 4.45 µg/mL) levels, consistent with an accelerated transition into the tissue remodeling phase.

Introduction

An incision wound is an open injury caused by a sharp object such as a knife or broken glass (Abdullah, 2022). Once the skin barrier is breached, the body responds with bleeding, coagulation, exposure to bacterial contamination, and localized cell death, all of which disrupt normal tissue function and make repair of the injured area necessary (Wahyuni *et al.*, 2022). This repair process, known as wound healing, unfolds through four overlapping phases – hemostasis, inflammation, proliferation, and remodeling – and its pace depends on variables such as the patient's age, general health, and the type of care the wound receives (Rodrigues *et al.*, 2019). Because these variables are not always controllable, choosing an effective and safe topical treatment is central to achieving timely healing.

Ointments remain one of the most common conventional wound treatments, valued for their semi-solid consistency and their capacity to shield the wound surface from infection. Many commercial

formulations, however, still rely on synthetic antibacterial agents; topical neomycin, for example, is a well-documented cause of allergic contact dermatitis and delayed skin irritation with repeated use (Gehrig & Warshaw, 2008). This drawback has directed research toward natural alternatives that are effective yet less likely to sensitize the skin, and collagen has emerged as one of the more promising candidates (Geahchan *et al*., 2022). As the principal structural protein of the extracellular matrix, collagen supports skin hydration and structural integrity, a benefit demonstrated even for collagen sourced from fish rather than mammals (Dondero *et al*., 2025). Collagen of marine origin is attractive for formulation work because it denatures at a comparatively low temperature, which simplifies its conversion into soluble peptide fractions during extraction (Jafari *et al*., 2020). Indonesia's abundant fishery waste adds a practical incentive for pursuing this route: converting scales, skin, and bone into collagen would supply a raw material for wound-care products while reducing the volume of processing by-products that would otherwise be discarded. Tilapia (*Oreochromis niloticus* L.) is a natural candidate for this purpose, given the scale of its production.

Tilapia is Indonesia's leading freshwater aquaculture species by production volume, reaching 1,300,529.23 tons in 2021 and continuing to rise each year, driven largely by growth in the fillet-processing industry (KKP, 2023). Fillet processing, however, leaves behind considerable waste, and scales are among the by-products that can be redirected into collagen extraction rather than discarded as an environmental burden (Romadhon *et al*., 2019). Tilapia scale collagen is predominantly type I collagen, the structural protein that forms the extracellular matrix and provides the tensile strength needed for wound closure (Hadiyantama *et al*., 2024). Romadhon *et al*. (2019) characterized collagen from tilapia bone, skin, and scale and confirmed that the scale-derived extract consists mainly of type I collagen fibers, with only minor residual hydroxyapatite likely attributable to incomplete demineralization during extraction rather than a defining feature of the extracted collagen itself. Building on this, Nugroho *et al*. (2022) formulated a tilapia skin collagen ointment for burn healing and found that a 15% collagen concentration produced the fastest wound closure among the concentrations tested, while a related hydrogel formulated from tilapia skin collagen likewise enhanced collagen deposition during burn treatment (Baydogan *et al*., 2023). Despite this early evidence, literature specifically addressing collagen-based ointments for incision wound healing – particularly formulations derived from tilapia scales rather than skin – remains limited, leaving room to examine whether the same benefit extends to a scale-derived extract and to a different wound type.

This study therefore evaluated the physical properties of a tilapia (*Oreochromis niloticus* L.) scale collagen extract ointment and examined its effect on wound closure, tissue protein content, and hydroxyproline content during incision wound healing in mice (*Mus musculus* L.).

Materials and methods

All reagents used in this study were of analytical grade. The principal materials were sodium hydroxide, glacial acetic acid, sodium chloride, and potassium bromide for collagen extraction and FTIR sample preparation; lanolin, petroleum jelly (Vaseline®), and methylparaben for ointment formulation; a commercial bioplacenton ointment as the positive-control treatment; and Chloramine-T, citrate buffer (pH 6), Ehrlich's reagent, and perchloric acid for the hydroxyproline assay (Solarbio, BC0170, China).

The study design used was a completely randomized design consisting of groups K- (no treatment), K+1 (bioplacenton), K+2 (ointment base), P1 (5% tilapia fish scale collagen extract ointment), P2 (10% tilapia fish scale collagen extract ointment), P3 (15% tilapia fish scale collagen extract ointment).

The experiment proceeded in the following order: sample collection, collagen extraction from tilapia scales, FTIR (*Fourier Transform Infrared*) characterization, ointment preparation, and evaluation of the ointment formulation. Test animals then received the assigned treatments for 14 days, during which incision wound closure, tissue protein content, and hydroxyproline content were assessed as the primary outcome measures.

1. Collection of samples

Tilapia scale samples were washed using cold water and then dried at room temperature. Afterward, 400 grams of the sample was weighed and soaked in a 0.1 N NaOH solution for 12 hours, changing the solution every 2 hours. The samples were then air-dried for 2 hours and rinsed with water until the pH of the rinse water reached neutral. Once the pH was near neutral, the samples were air-dried again to reduce the water content. They were then dried using a food dehydrator set at 60°C until completely dry. Once

completely dry, the tilapia scales were ground using a grinder to obtain a powdered scale sample (Nugroho *et al.*, 2022).

2. Collagen extraction

The powdered scale samples from the preparation stage were then extracted using a 0.5 M acetic acid solution for 48 hours, stirring every four hours (Romadhon *et al.*, 2019). The extraction was then filtered using a muslin filter cloth (100 mesh). The filtrate was then soaked in NaCl to a concentration of 1 M. This soaking was carried out for 12 hours, followed by centrifugation for 1 hour at 6000 rpm and 4°C (Zaelani *et al.*, 2019).

The resulting precipitate was then rinsed with distilled water until the pH of the rinse was neutral. The wet collagen obtained was then dried using a food dehydrator set at 60^{0}C for 12 hours and then ground using a mortar and pestle to obtain powdered collagen extract (Nugroho *et al.*, 2022).

3. Calculation of the yield

The yield of tilapia fish scale extract is calculated based on the percentage of dry collagen weight obtained compared to the dry sample weight before extraction (Agustina *et al.*, 2015).

The following is the yield calculation formula:

$$\text{Yield } \% = \frac{\text{W final (g)}}{\text{W initial (g)}} \times 100\% \quad \text{.......(1)}$$

(Paudi *et al.,* 2020)

4. Characterization with FTIR (*Fourier Transform Infrared*)

Characterization of tilapia fish scale extract was done using the FTIR. The FTIR test process begins by taking a small amount of dry collagen and mixing it with 0.25 grams of KBr. This mixture is ground using a mortar and placed in a pallet, then pressed to form a transparent layer. The sample is then placed in a sample holder and tested at a wavelength range of 4000-400 cm^{-1} (Ramdhani & Ariani, 2016).

5. Preparation of ointment

All ingredients were weighed according to the formulation. Lanolin was first combined in a mortar with the tilapia scale collagen extract at each of the three concentrations. Petroleum jelly (Vaseline®) and methylparaben were then added, and the mixture was ground until homogeneous before being transferred into an ointment jar. The formulation used to prepare 50 g of ointment is shown in Table 1.

Table 1. Tilapia Fish Scale Collagen Ointment Preparation Formulation

No.	Ingredient (g)	P1	P2	P3
1.	Collagen extract from fish scales	2.5	5	7.5
2.	Lanoline	22.5	22.5	22.5
3.	Methyl paraben	6	6	6
4.	Vaseline	19	16.5	14

(Nugroho *et al.*, 2022).

6. Evaluation of ointment formulation

Evaluation of the ointment formulation encompassed organoleptic. homogeneity. pH. adhesion. spreadability. and emulsion-type testing. Although the base is predominantly oleaginous. the aqueous collagen extract solution incorporated into the formulation introduces a dispersed phase. so, an emulsion-type test remains informative for characterizing how the preparation is expected to release its active component. The standard parameters against which the preparation was evaluated are summarized in Table 2.

Table 2. Evaluation Parameter Standards for Ointment Formulation

No.	Test Parameters		Test Parameter Standard
1.	Organoleptic	Aroma	Aroma Characteristics: Neutral. fishy. fresh. and synthetic Absence of rancid aroma
		Color	White. Yellow. and Brown
		Texture	Liquid: Low viscosity Thick: High viscosity
2.	Homogenity		Homogeneous: No coarse grains/particles

No.	Test Parameters	Test Parameter Standard
		Non-homogeneous: Has coarse grains/particles
3.	pH	4.5-6.5
4.	Adhesiveness	> 1 minute
5.	Spreadability	5-7 cm
6.	Cream type	Oil emulsion: Methylene blue is evenly distributed Water emulsion: Methylene blue only appears in spots

(Mardiansyah *et al.*, 2024).

7. Observation of incision wound closure

A number of male Swiss mouse (Three months age. average body weight 25 g). was used in current study. Wound in the dorsal body was made by using sharp scalpel with 1 cm in length and 1 mm in depth. The treatment of ointment was administered for 14 days. and wound healing was observed over the 14 days. Wound healing was observed on days 0. 2. 4. 6. 8. 10. 12. and 14. Wound healing was observed by measuring the length of the incision using a caliper and photographing the wounds on the mice. The obtained data was then substituted into an equation to determine the percentage of wound closure. The equation used is as follows:

$$P\% = \frac{(d_0)-(d_x)}{(d_0)} \times 100\% \quad (2)$$

Description:
P%: Percentage of wound healing
d_0: Length of wound on day 0
d_x: Length of wound on day x

(Susanto *et al.*, 2023).

8. Protein assay

This process begins by weighing 0.05 grams of skin tissue. then adding 300 µL of PBS solution so that the protein in the tissue can be extracted. Next. homogenization is carried out using a homogenizer at a speed of 3000 rpm for 30 seconds. After that. the sample is centrifuged at a speed of 6000 rpm at room temperature for 10 minutes to separate the supernatant liquid containing protein. Then. 150 µL of the supernatant is mixed with 1200 µL of Lowry B reagent and waited for 10 minutes at room temperature. Next. 150 µL of Lowry A reagent is added and then homogenized and left for 20 minutes until a color change occurs to purplish blue (bluish color). which indicates an interaction between the protein and Lowry reagent. Then. the absorbance is measured using a spectrophotometer at a wavelength of 600 nm (Wati. 2024).

9. Hydroxyproline assay

A 0.05gram sample was weighed and cut into small pieces to facilitate the extraction process. then placed in a glass tube. Next. the sample was incubated at 60°C for 24 hours. After 24 hours. the sample was left to cool to room temperature. When the sample reached room temperature. 3 mL of 6N HCl was added to the sample and hydrolyzed for 4 hours at 130°C for 4 hours. The hydrolysate was then allowed to cool to room temperature. then 2 mL was taken and placed in a microcentrifuge tube and centrifuged for 5 minutes at 10.000 rpm. The supernatant obtained was then placed in a glass tube and evaporated at 60°C for 40 minutes. After that. 500 µL was taken and transferred into an Eppendorf tube. Then. 30 µL of Chloramine-T solution was added. as well as 470 µL of citrate buffer pH 6. and vortexed until homogeneous. Next. it was incubated at room temperature for 20 minutes. After that. 250 µL of 0.4 M $HClO_4$ solution was added and homogenized. Then. 250 µL of Ehrlich solution was added and homogenized again. Next. the sample was incubated at 60°C for 90 minutes. The reaction results will form a precipitate and centrifuged at 3000 rpm for 5 minutes. Then. the absorbance was measured using a spectrophotometer at a wavelength of 557 nm (Gupta *et al.*, 2016).

10. Data analysis

The research data obtained from the evaluation of ointment preparations were analyzed descriptively. while the measurement data of the percentage of wound healing. protein content. and hydroxyproline content were analyzed using GraphPad Prism and SPSS 22 software. Normality and homogeneity tests were performed using GraphPad Prism. Data originating from normally distributed data were then analyzed using One-way ANOVA to determine whether there was an effect of tilapia fish scale collagen administration on wound healing. Then. to see significant differences between groups. it was continued with the Tukey test with a significance of P <0.05. Data originating from non-normally distributed and non-homogeneous data were then analyzed using the Kruskal Wallis test and continued with the Duncan test with a significance of P <0.05 to determine significant differences. To see the effect of observation days on wound healing. data originating from non-normally distributed and non-homogeneous data were then carried out the Friedman test and continued with the Wilcoxon test with a significance of P <0.05. Then. to determine the correlation between parameters. a correlation test was carried out using SPSS 22.

Results and discussion

1. Calculation of the yield

Collagen extraction from tilapia scales with an initial weight of 307 grams yielded 150 grams of wet collagen. and after drying. 18 grams of dry collagen was obtained. Calculations showed that the wet collagen yield reached 48.86%. while the dry collagen yield was 5.86%. This dry collagen yield is relatively higher than previous research reports. Romadhon *et al.* (2019) reported tilapia collagen extraction yields of 0.53% from bones. 0.63% from scales. and 0.94% from skin.

The comparatively high yield obtained here is likely attributable to particle-size reduction: the dried scales were ground and sieved into a fine powder before extraction. which increased the surface area available for contact with the solvent. Scales ground into smaller particles have a larger surface area in contact with the solvent. accelerating the diffusion of acetic acid into the scale tissue and maximizing collagen release. This principle aligns with the research of Tambun *et al.* (2016) stated that particle size influences extraction results. where the smaller the particle size. the greater the surface area of the substance. thus accelerating the solubility of the substance in the extraction process. The acetic acid concentration and the solid-to-liquid ratio applied in the present protocol were fixed rather than systematically varied. and it remains possible that yield could be further improved by testing a range of values for these two parameters in future work.

2. Characterization with FTIR (*Fourier Transform Infrared*)

Characterization was performed after collagen extraction from tilapia scales to ensure the presence of collagen formed from the extraction. FTIR analysis was used to identify the main functional groups present in the collagen samples extracted from the fish scales. The FTIR spectra are presented in Fig. (Figure 1; Table 3).

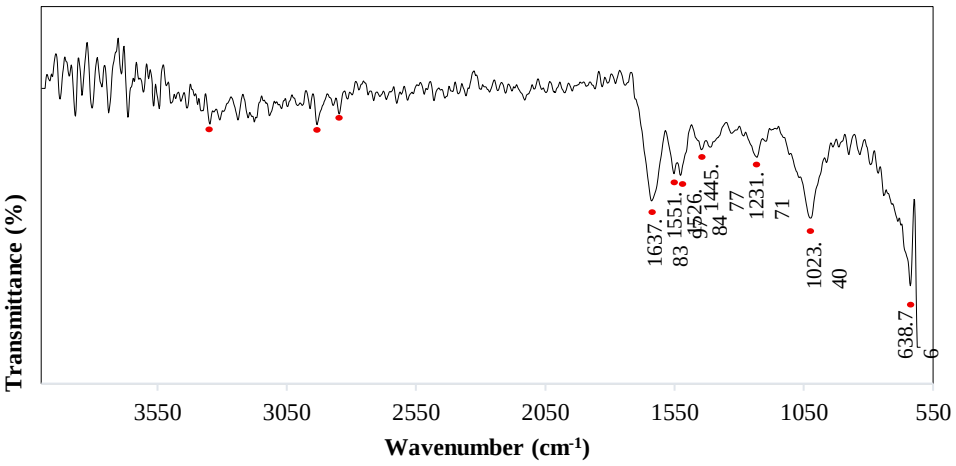

Figure 1. FTIR Spectrum of Tilapia Fish Scale Extract

Based on Figure 1. the FTIR spectrum of tilapia fish scale collagen is in the range of 600 – 4000 cm^{-1}. This spectrum shows several peaks that are indicators of collagen characteristics. namely at wave numbers 3542.21; 3346.98; 2032.88; 1637.82; 1526.84; 1023.40; 638.76; 1231.71; 1445.77; 2847.12; and 1551.97. These five wave peaks are evidence of the presence of collagen. namely amide A. amide B. amide I. amide II. and amide III. The amide A peak is in the wave range In addition to these five peak points. there are other peak points produced from the sample. The detection results show a peak at 3542.21 cm^{-1} as a hydroxyl group. The peak at 1023.40 cm^{-1} is the alcohol group. The peak at 638.76 cm^{-1} is the fingerprint region. The peak at 1445.77 cm^{-1} is the methylene group. The peak at 2847.13 cm^{-1} is the protein hydrocarbon chain.

Table 3. FTIR Analysis Results of Tilapia Fish Scale Collagen Extract

No.	Gelombang (cm^{-1})	Literatur (cm^{-1})	Tampilan Pita	Gugus	Jenis Senyawa
1.	3542.21	3400 (Mberato *et al.*, 2020)	Wide. overlapping. weak	O – H	Hydroxyl group
2.	3346.98	3360 – 3310 (Mberato *et al.*, 2020)	Medium to wide. strong	N – H	Amida A
3.	2932.89	2935 – 2915 (Mberato *et al.*, 2020)	Sharp to narrow. strong	C – H	Amida B
4.	1637.83	1690 – 1600 (Mberato *et al.*, 2020)	Sharp. strong	C = O	Amida I
5.	1526.84	1575 – 1480 (Mberato *et al.*, 2020)	Narrow to medium. medium	N – H	Amida II
6.	1023.40	1020 (Safithri *et al.*, 2019)	Slightly wide. medium	C – O	Alcohol group
7.	638.76	700 (Mberato *et al.*, 2020)	Overlapping. weak	N – H	Fingerprint region
8.	1231.71	1309 – 1229 (Mberato *et al.*, 2020)	Sharp to medium. strong	C – N	Amida III
9.	1445.77	1440 (Mberato *et al.*, 2020)	Medium or sharp. medium	C – H	Methylene group
10.	2847.13	2845 (Safithri *et al.*, 2019)	Sharp to medium. medium	C – H	Protein carbon chain
11.	1551.97	1575 – 1480 (Mberato *et al.*, 2020)	Sharp to medium. medium	N – H	Amida II

The Amide A band detected at 3347 cm^{-1} reflects the presence of hydrogen bonds within the protein structure. which are crucial for maintaining the stability of the collagen triple helix. This stability enables collagen to interact with fibroblasts and supports extracellular matrix formation during wound healing. Consistent with Oslan *et al.* (2022). the detection of Amide A serves as a reliable indicator of collagen quality. as these hydrogen bonds are directly linked to the preservation of its triple helix structure. Similarly. the Amide B band detected at 2933 cm^{-1} corresponds to C–H bonds derived from amino acid residues. particularly proline and hydroxyproline. which sustain the collagen polypeptide framework. Its presence confirms that the triple helix remains intact. reinforcing the identification of the protein as collagen. In agreement with Slimane & Sadok (2018) and Oslan *et al.* (2022). Amide B is recognized as a distinctive marker of fish collagen and provides evidence that the extraction process preserves molecular integrity.

The Amide I band detected at 1638 cm^{-1} indicates the stretching vibration of the carbonyl group (C=O) of the peptide bond and is the most important marker in the FTIR analysis of collagen. Its presence confirms that the extraction process successfully maintains the basic structure of collagen. especially the triple helix that is characteristic of this protein. In line with the study of Oslan *et al.* (2022). Amide I is considered a primary indicator of fish collagen quality because it directly reflects the integrity of the peptide bond. The Amide II bands detected at 1552 and 1527 cm^{-1} show a combination of N–H and C–N vibrations. in accordance with the typical characteristics of this band. Its presence confirms that the collagen

peptide bond remains intact. thus, maintaining the protein structure. In line with Baydogan *et al.* (2023). Amide II is an important indicator of peptide integrity in fish collagen. including in biomedical applications such as hydrogels for burn therapy. The Amide III band detected at 1232 cm^{-1} exhibits C–N stretching and N–H bending vibrations. consistent with the typical characteristics of this band. This peak is an important marker for the presence of the collagen triple helix structure. as well as evidence that the extraction process does not damage the protein's integrity. In line with Mufidah *et al.* (2024). Amide III is considered a primary indicator of fish collagen quality because it directly reflects the stability of the triple helix structure.

Overall. the FTIR results indicate that the fish scale collagen tested has all the typical collagen bands. namely Amide A. Amide B. Amide I. Amide II. and Amide III. These five amide bands correspond to the wavenumber range reported in the literature. thus, confirming that the protein produced is collagen. Additionally. the presence of extra bands further strengthens the results of the spectral interpretation. Thus. it can be concluded that the extraction process successfully produced collagen with structural characteristics that are still maintained and in accordance with standard collagen. The results of the FTIR analysis are presented in Table 3.

3. Evaluation of oinment formulation

Based on the evaluation of ointment preparations that have been carried out. through organoleptic tests carried out by 35 probands. it is known that the color aspect of the ointment is in accordance with the color code reference given in the yellow ocher earth tone color gradation identified in the color code #EDE8CA (light khaki) for the color of collagen extract ointment with a concentration of 5%. and the color code #D8CD8D (pale goldenrod) for the color of collagen extract ointment with a concentration of 10% and 15%. This shows a tendency for color intensity to increase as collagen concentration increases. Furthermore. in terms of aroma. the three collagen extract ointment preparations are neutral and do not disturb the sense of smell. and there is no rancid aroma. Then. in terms of texture. it shows that the ointment preparations at three concentrations have good viscosity with a semi-solid dosage form. Through the results of organoleptic tests. it shows that the ointment preparations have met national quality standards in accordance with the Indonesian Ministry of Health No. HK.395/MENKES/SK/X/1979 states that ointment is a semi-solid preparation for the skin with a uniform color. normal aroma and not rancid (Kemenkes. 1979).

Furthermore. through homogeneity testing. it was found that the ointment preparation had a good level of homogeneity. this was indicated by consistent color uniformity. both at the top. middle. and bottom of the ointment pot. Through pH testing. the results showed that the pH of the three ointment preparations was 6.5. This indicates that the tilapia fish scale collagen extract ointment has met the national standard based on SNI 16-4380-1996. which states that a good ointment preparation has a pH value ranging from 4.5-6.5. This value is within the physiological pH range of human skin. which is between 4.5-6.5. which is considered optimal for maintaining skin moisture and preventing skin irritation (Latifah *et al.*, 2022). Based on adhesion and spreadability tests. it was shown that the tilapia fish scale collagen extract ointment had an adhesive power for 5-5.85 minutes. This adhesive power indicates the ointment's ability to maintain adhesion to the skin surface well. At a 5% concentration. the ointment had a spreadability of 5.85 cm. The 10% ointment concentration had a spread of 5.1 cm. The 15% ointment concentration had a spread of 5 cm. This indicates that the tilapia scale collagen extract ointment meets national standards based on SNI 16-4380-1996. which states that a good ointment preparation has an adhesive strength of more than 1 minute with a spread of more than 3 cm. Finally. the emulsion-type test showed that the tilapia scale collagen extract ointment behaves as a water-in-oil preparation: a methylene blue staining test showed the oil-soluble dye spreading evenly through the preparation. consistent with an oleaginous continuous phase. This type has occlusive properties that can form a protective layer on the skin surface. thereby reducing water evaporation from wound tissue and maintaining skin moisture (Cahya *et al.*, 2022).

4. Observation of incision wound closure

The wound healing process in mice was observed for 14 days. by measuring the length of the wound every 2 days. on days 0. 2. 4. 6. 8. 10. 12. and 14 (Figure 2). then the data was entered into equation 1. Data analysis of the average percentage of wound healing was carried out using the GraphPad Prism application and is presented in Table 4.

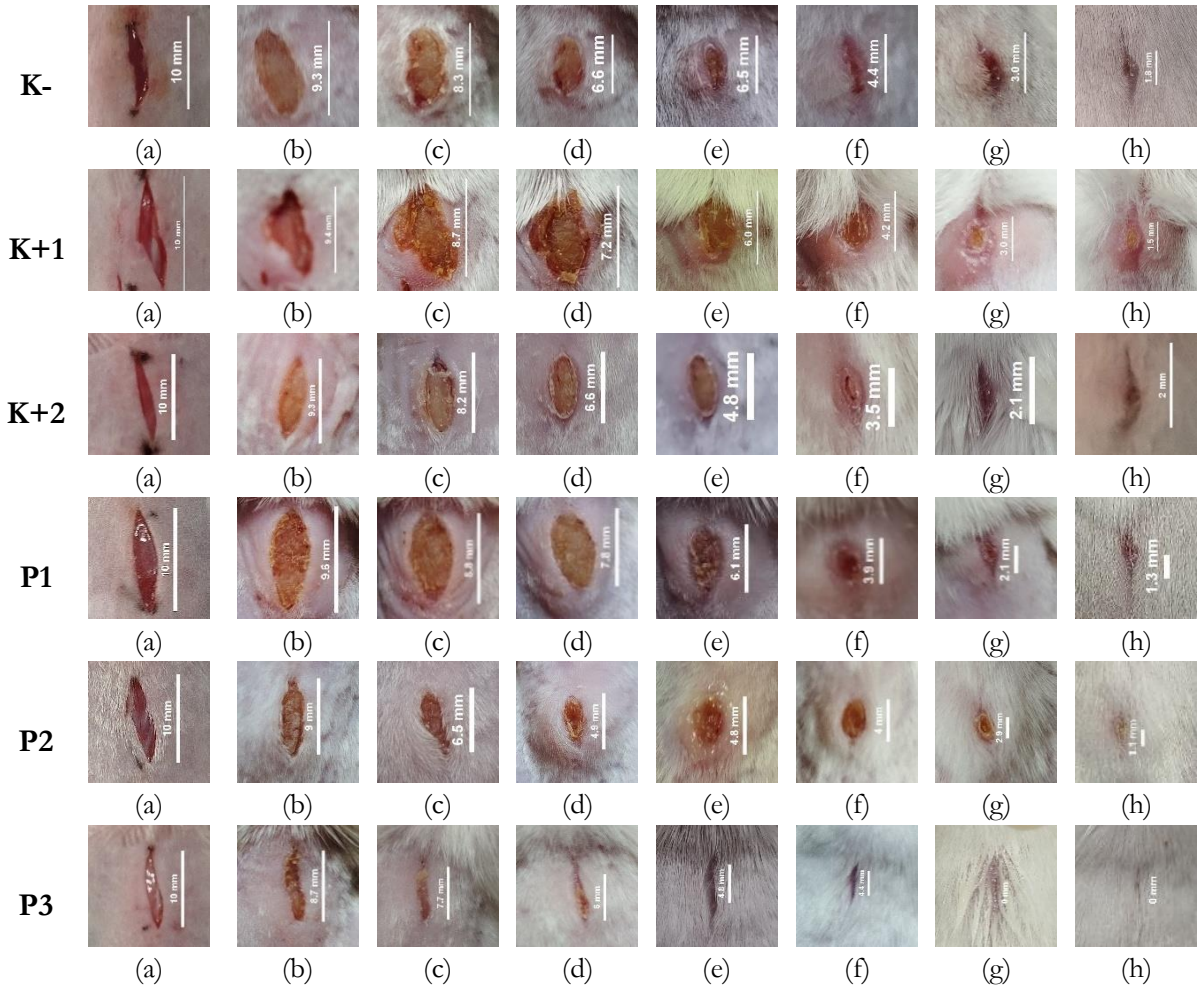

Figure 2. Observation of Incision Wounds Closure in Mice on Days 0. 2. 4. 6. 8. 10. 12. and 14 Information: (a) Day 0. (b) Day 2. (c) Day 4. (d) Day 6. (e) Day 8. (f) Day 10. (g) Day 12. & (h) Day 14. Rows correspond to treatment groups: K- (untreated). K+1 (bioplacenton). K+2 (ointment base only). P1 (5% collagen ointment). P2 (10% collagen ointment). and P3 (15% collagen ointment).

Based on Figure 2. it can be seen that the wound healing process in mice occurs gradually from day 0 to day 14. as shown in the macroscopic observations of incisional wounds in each group. On day 0. all groups of mice showed fully open wounds. Over time. there was a progressive increase in wound closure. with the peak of healing reached on day 14. At the final observation point (day 14). the mice in the P3 treatment group (15% tilapia fish scale collagen extract ointment) showed the fastest healing rate compared to the other groups. On day 14. wounds were still visible in several other groups. The wound length in the K- group was 1.8 mm. which was the longest among the groups. The wound length in the K+1 group was 0.6 mm. In the K+2 group. it was 2 mm. In the P1 group. the wound length was 1.3 mm. In the P2 group. it was 1.1 mm. The shortest wound length was found in the P3 group. at 0 mm. Based on visual appearance. the group of mice with the fastest wound healing process was the P3 group.

Table 4. Mean Percentage of Incision Wound Closure in Mice by Treatment Group and Observation Day

Ket.	Mean Percentage of Wound Closure by Observation Day (%)						
	2	4	6	8	10	12	14
K-	$_{1}12.0\pm1.67^{a}$	$_{2}29.60\pm3.47^{a}$	$_{3}44.60\pm4.09^{a}$	$_{4}59.40\pm4.92^{a}$	$_{5}64.00\pm4.35^{a}$	$_{6}84.20\pm4.24^{a}$	$_{6}90.40\pm4.26^{a}$
K+1	$_{1}9.6\pm0.67^{a}$	$_{2}24.80\pm1.46^{a}$	$_{3}44.20\pm2.51^{a}$	$_{4}54.60\pm2.15^{a}$	$_{5}59.20\pm0.58^{a}$	$_{6}91.80\pm5.02^{a}$	$_{6}91.80\pm5.02^{a}$
K+2	$_{1}5.8\pm1.24^{a}$	$_{2}18.00\pm1.76^{a}$	$_{3}31.20\pm2.95^{a}$	$_{4}50.40\pm3.98^{a}$	$_{5}59.60\pm3.76^{a}$	$_{6}87.40\pm3.80^{a}$	$_{7}87.40\pm3.80^{a}$
P1	$_{1}11.0\pm2.60^{a}$	$_{2}22.40\pm3.77^{a}$	$_{3}40.60\pm5.51^{a}$	$_{4}50.20\pm4.10^{a}$	$_{5}64.40\pm1.43^{a}$	$_{6}80.60\pm2.58^{a}$	$_{7}93.60\pm3.20^{a}$

P2	$_{1}$7.60±0.87^{a}	$_{2}$24.80± 1.65^{a}	$_{3}$42.60±3.34^{a}	$_{4}$57.80±2.43^{a}	$_{5}$66.80± 1.62^{a}	$_{6}$83.60±4.40^{a}	$_{6}$95.80±1.80^{a}
P3	$_{1}$8.80±1.49^{a}	$_{2}$25.40± 1.56^{a}	$_{3}$46.60±1.86^{a}	$_{4}$57.80±1.46^{a}	$_{5}$66.60±2.48^{a}	$_{6}$88.20±1.80^{a}	$_{7}$98.20±1.80^{a}

Description: K- (Group without treatment). K+1 (Group given bioplacenton). K+2 (Group given ointment base). P1 (Group given 5% tilapia fish scale collagen extract ointment). P2 (Group given 10% tilapia fish scale collagen extract ointment). P3 (Group given 15% tilapia fish scale collagen extract ointment). The mean value ± SE and followed by the same superscript (a) in one column indicates no significant difference and the subscript numbers (1. 2. 3. 4. 5. 6. 7) in one row indicate a significant difference with a significance value ($p < 0.05$).

However. based on Table 4. the results of statistical tests conducted on the control and treatment groups on all observation days show the same superscript (a). This indicates that although visually there are differences in wound healing conditions in mice. when statistical tests were conducted there were no significant differences between each group. In other words. although the wounds were treated differently (no treatment. bioplacenton. ointment base. and collagen extract ointment concentrations of 5%. 10%. & 15%). statistical analysis of the macroscopic data of the wounds showed healing at a relatively similar rate. This condition can occur due to several possible factors. including the condition and ability of each skin. the age of the test animal. and the type of wound applied. This pattern is consistent with Rodrigues et al. (2019). who note that in rodent models. wound closure is driven substantially by skin contraction rather than by the treatment applied. which can obscure differences between groups; visual assessment alone therefore has limited resolving power. and a molecular or histological readout would provide firmer evidence of a treatment effect.

In contrast. statistical tests conducted on the differences in treatment days in each group showed different subscripts. on days 0 to 14. respectively (1. 2. 3. 4. 5. 6. 7). This indicates a significant difference between observation days in each group. This significant difference indicates that the length of observation time has a greater influence on the healing process when compared to the treatment given. Furthermore. the significance found between observation days within each group agrees with Meyers *et al.* (2020). who noted that macroscopic wound assessment is generally more sensitive to the passage of time than to differences between treatments. making the observation day the dominant factor behind what a purely visual analysis can detect. This is in line with the biological mechanism of wound healing which occurs in stages and overlaps. consisting of the hemostasis phase. inflammation phase. proliferation phase. and remodeling phase.

5. Protein and hydroxyproline assay

Measurement of protein levels and hydroxyproline levels was carried out on the 14th day. then the data was analyzed using the GraphPad Prism and SPSS applications to obtain the results as in (Table 5).

Table 5. Tissue Protein and Hydroxyproline Content in Mice on Day 14 by Treatment Group

Group	Protein content (μg/mL)	Hydroxyproline content (μg/mL)
K-	804.8 ± 7.35^{a}	200.0 ± 1.77^{a}
K+1	452.0± 5.77^{c}	132.1 ± 11.0^{b}
K+2	621.4± 3.38^{b}	173.6 ± 3.72^{a}
P1	585.9± 18.37^{b}	143.8 ± 7.26^{b}
P2	478.7± 6.93^{c}	128.2 ± 0.67^{b}
P3	397.0± 8.55^{d}	82.05 ± 4.45^{c}

Description: K- (Group without treatment). K+1 (Group given bioplacenton). K+2 (Group given ointment base). P1 (Group given 5% tilapia fish scale collagen extract ointment). P2 (Group given 10% tilapia fish scale collagen extract ointment). P3 (Group given 15% tilapia fish scale collagen extract ointment). Mean values ± SE and followed by superscript letters (a. b. c. d) for differences between groups in the same column with a significance value ($p < 0.05$).

Based on Table 5. tissue protein levels differed significantly between the treatment groups and the untreated control. Mice in the K- group. which received no treatment. had the highest protein content at 804.8 ± 7.35^{a} μg/mL. consistent with inflammation and tissue regeneration proceeding without any external intervention. This elevated level suggests that incision wounds in the K- group remained in an early

proliferative stage. since no treatment was present to hasten the transition into remodeling; a comparable association between tissue protein content and active extracellular-matrix synthesis during proliferation has been reported by Mahmoud *et al.* (2024). It should be noted that the Lowry assay used here quantifies total soluble protein rather than fibroblast number directly. so a marker such as PCNA or Ki-67 immunostaining would be needed to confirm proliferative activity specifically. In the K+2 (ointment base) and P1 (5% collagen ointment) groups. protein levels were 621.4 ± 3.38^{b} and 585.9 ± 18.37^{b} µg/mL. respectively; relative to the K- group. both values are consistent with an active proliferative phase in which collagen synthesis continues at a high rate. Mice in the K+1 (bioplacenton) and P2 (10% collagen ointment) groups had protein levels of 452.0 ± 5.77^{c} and 478.7 ± 6.93^{c} µg/mL. respectively. with no significant difference between them. indicating that the 10% ointment regulated tissue protein to an extent comparable with bioplacenton. Group P3 (15% collagen ointment) showed the lowest protein level. at 397.0 ± 8.55^{d} µg/mL. significantly different from every other group; this may indicate that. at this concentration. the healing process had already advanced into a later. more resolved phase in which protein turnover slows as inflammation subsides. This pattern is consistent with Alberts *et al.* (2025). who reported that collagen-based dressings can support remodeling by lowering inflammatory protein levels while promoting new collagen deposition. and with Anggraeni (2023). who observed that tissue protein demand rises during the early phase of wound healing before falling as the tissue matures and stabilizes.

Based on Table 5. hydroxyproline levels likewise differed significantly between the treatment groups and the untreated control. Mice in the K- group showed the highest level. 200.0 ± 1.77^{a} µg/mL. indicating that collagen synthesis was still proceeding through natural fibroblast activity in the absence of any treatment; Ding *et al.* (2025) similarly reported that collagen synthesis peaks during the proliferation phase. which can persist until around day 21 before declining as remodeling begins. Mice in the K+2 group had a hydroxyproline level of 173.6 ± 3.72^{a} µg/mL. not significantly different from the K- group. suggesting that the ointment base alone did little to advance healing beyond the stage reached without treatment. The K+1. P1. and P2 groups had hydroxyproline levels of 132.1 ± 11.0^{b}. 143.8 ± 7.26^{b}. and 128.2 ± 0.67^{b} µg/mL. respectively. with no significant differences among them. indicating that proliferation was still active in these groups but had begun to give way to remodeling relative to K- and K+2. Group P3 (15% collagen ointment) showed the lowest hydroxyproline level. 82.05 ± 4.45^{c} µg/mL. significantly lower than every other group. This decline points to a more advanced remodeling stage in which collagen turnover slows as inflammation resolves. consistent with Ding *et al.* (2025). who linked falling hydroxyproline to tissue stabilization and reduced fibroblast activity. and with Utami *et al.* (2025). who associated declining hydroxyproline with more effective wound healing. Dira *et al.* (2018) similarly noted that. because hydroxyproline is an amino acid largely specific to collagen. its level serves as a practical indicator of collagen content across the healing process.

Conclusion

Based on the research conducted. it can be concluded that tilapia scale collagen extract ointment at concentrations of 5%. 10%. and 15% has good physical properties and meets the standards for topical preparations. Observations on all groups of test animals showed a gradual wound closure process up to day 14 without significant differences. However. molecular analysis of protein and hydroxyproline levels showed that tilapia scale collagen extract ointment at a concentration of 15% has the potential to support the wound healing process.

Author Statements

Acknowledgements and funding statements: The authors also express their gratitude to all those who assisted in this research. although their names cannot be included as authors. They also express their gratitude for the facilities provided by the Biology Department Laboratory. Faculty of Mathematics and Natural Sciences. Mulawarman University. Samarinda. East Kalimantan. Indonesia.

Competing of interest: The authors declare no conflict of interest related to this research.

Author's contributions: Sari: Conceptualization. methodology. investigation. data curation. writing. and original draft. Rudy Agung Nugroho: Methodology. supervision. writing. review. and editing. Retno Aryani: Supervisor. methodology. and resources.

Generative AI: This article was written using AI. namely Microsoft Copilot and ChatGPT for paraphrasing and text editing.
Data availability: Not applicable.

References

Abdullah. D. (2022). *Penyembuhan Luka Sayat pada Mencit Melalui Pemberian Gel Kefir*. Indramayu: Penerbit Adab.

Alberts. A.. Bratu. A. G.. Niculescu. A.-G.. & Grumezescu. A. M. (2025). Collagen-Based Wound Dressings: Innovations. Mechanisms. and Clinical Applications. Gels. 11(4): 271. https://doi.org/10.3390/gels11040271

Afriani. Y.. Rahayu. R.. & Santoso. P. (2024). Black Soldier Fly (*Hermetia illucens* L.) Larvae Oil Oinment to Accelerate Incision Wound Healing in Mice. *Jurnal Biologi Tropis*. 24(3): 292-297. http://doi.org/10.29303/jbt.v24i3.7272

Agustina. S. S. (2015). Isolasi Kitin. Karakterisasi. dan Sintesis Kitosan dari Kulit Udang. *Jurnal Kimia*. 9(2): 271-278. https://doi.org/10.24843/JCHEM.2015.v09.i02.p19

Anggraeni. F. (2023). *Gambaran Penyembuhan Luka Pada Pasien Pasca SC di Poli Kandungan RS Sahabat Sukorejo Kabupaten Pasuruan* (Skripsi). Universitas Bina Sehat PPNI.

Baydogan. B.. Kucuk. A.. Kozan. B.. Erdal. M.. Abas. B. I.. & Cevik. O. (2023). Hydrogels Made With Tilapia Fish Skin Increase Collagen Production And Affect MMP-2/MMP-9 Enzymes In Burn Treatment. *Biochem*. 5(2): 1-16. https://doi.org/10.3390/biochem5020008

Cahya. E. R. P.. Ekowati. D.. & Ningsih. D. (2022). Formulating Oil Emulgel of Nyamplung (*Calophyllum inophyllum* L.) with Variations of HPMC and Activity Testing for Incision in Rabbit New Zealand. *Indonesian Journal of Pharmaceutical Education*. 2(3): 161-175. https://doi.org/10.37311/ijpe.v2i3.15455

Ding. P.. Sun. Y.. Jiang. G.. & Nie. L. (2025). Hydroxyproline-Modified Chitosan-Based Hydrogel Dressing Incorporated with Epigallocatechin-3-Gallate Promotes Wound Healing Through Immunomodulation. *Gels*. 11(9): 1-22. https://doi.org/10.3390/gels11090732

Dira. M. A.. Sari. D. P.. & Sari. R. P. (2018). Pengaruh Pemberian *Alfa Mangostin* Terhadap Kadar Hidroksiprolin Pada Hari Ke-10 Sesudah Luka Pada Tikus Putih Jantan. *Jurnal Ilmiah Mahasiswa Fakultas Kedokteran Universitas Andalas*. 7(4): 1–8. DOI:10.36434/scientia.v8i1.143

Dondero. L.. De Negri Atanasio. G.. Lertora. E.. Tardanico. F.. Demori. I.. Storace. P.. Allaria. G.. Rispo. F.. Robino. F.. Perata. E.. Zanotti-Russo. M.. Ferrando. S.. & Grasselli. E. (2025). Effects of a single application of hydrolyzed fish collagen on dermal protein expression and tissue architecture in human skin models. Scientific Reports. 15: 11372. https://doi.org/10.1038/s41598-025-11372-5

Geahchan. S.. Baharlouei. P.. & Rahman. A. (2022). Marine Collagen: A Promising Biomaterial for Wound Healing. Skin Anti-Aging. and Bone Regeneration. *Marine drugs*. 20(61): 1-16. https://doi.org/10.3390/md20010061

Gehrig. K. A.. & Warshaw. E. M. (2008). Allergic contact dermatitis to topical antibiotics: Epidemiology. responsible allergens. and management. Journal of the American Academy of Dermatology. 58(1): 1-21. https://doi.org/10.1016/j.jaad.2007.07.050

Gupta. R.. Garg. A.. Sharma. P.. & Pandey. P. (2016). Wound Healing and Antioxidant Effect of *Calliandra haemotocephala* Leaves on Incision and Excision Wound Models. *Asian Journal of Pharmacy and Pharmacology*. 2(2): 34 – 39.

Meyers. D. S. M.. Andrade. T. A. M.. Caetano. G. F.. Guimaraes. F. R.. Leite. M. N.. Leite. S. N.. & Frade. M. A. C. (2020). Experimental Models and Methods For Cutaneous Wound Healing. *International journal of experimental pathology*. 101(1):21–37. https://doi.org/10.1111/iep.12346

Hadiyantama. H.. Agustini. L.. Sujipto. Komaratih. E.. Zuhria. I.. Lestari. P.. & Ridholia. (2024). The Role of α-SMA. Type 1 Collagen. and Myofibroblasts in Cicatrizing Conjunctivitis by Alkali Chemical Trauma. *Pharmacognosy Journal*. 16(3): 591-596. https://doi.org/10.5530/pj.2024.16.92

Jafari. H.. Lista. A.. Siekapen. M. M.. Ghaffari-Bohlouli. P.. Nie. L.. Alimoradi. H.. & Shavandi. A. (2020). Fish collagen: Extraction. characterization. and applications for biomaterials engineering. Polymers. 12(10): 2230.

Kementerian Kelautan dan Perikanan (KKP). (2023). *Produksi Perikanan Kelautan dan Perikanan.* Jakarta: Direktorat Usaha dan Investasi Kementerian Kelautan dan Perikanan.

Kementerian Kesehatan Republik Indonesia (Kemenkes RI). (1979). *Farmakope Jilid 3*. Jakarta: Kementerian Kesehatan Republik Indonesia.

Latifah. S. L.. Pudjono. & Rosmi. R. F. (2022). Formulasi dan Evaluasi Mutu Fisik Sediaan Body Scrub Cream Varietas Ubi Jalar dalam Fase Air dan Minyak. *Pharmacy Peradaban Journal.* 2(1): 20-32. e-ISSN: 2776-6241.

Mahmoud. A. R. H.. Taha. N. M.. Mohamed. E. H.. & Abdelgayed. M. H. (2024). Factors Affecting Wound Healing Post Major Surgeries in Inensive Care Units. *Cuest. Fisioter.* 53(3): 6295-6308. 10.48047/jocaaa.2024.33.06.131

Mardiansyah. F.. Andrie. M.. & Taurina. W. (2024). Formulasi Sediaan Salep Kombinasi Fase Ekstrak Ikan Gabus (*Channa striata*) dan Madu Kelulut (*Heterotrigona itama*) Menggunakan Butil Hidroksil Toluen dan Vitamin C Sebagai Antioksidan. *Journal Pharmacy of Tanjungpura.* 2(1): 1-15. https://jurnal.untan.ac.id/index.php/JPOP

Mberato. S. P.. Rumengan. I. F. M.. Warouw. V.. Wulur. S.. Rumampuk. N. D. T.. Undap. S. L.. Suptijah. P.. & Luntungan. A. H. (2020). Penentuan Struktur Molekul Kolagen Sisik Ikan Kakatua (*Scarus* sp) Berdasarkan Serapan Molekul Terhadap Gelombang FTIR (*Fourier-Transform Infrared Spectroscopy Analysis). Jurnal Pesisir dan Laut Tropis*. 8(1): 7-14. https://doi.org/10.35800/jplt.8.1.2020.27285

Mufidah. A.. Rahayu. W. S.. & Utami. P. I. (2024). Narrative review: Extraction and characterization of collagen from various fish species. *Pharmacon: Jurnal Farmasi Indonesia*. 21(2): 198-205. https://doi.org/10.23917/pharmacon.v21i2.5391

Nugroho. R. T.. Saputra. G.. Aini. A. N.. Dewia. A. A.. & Tarigan. I. L. (2022). Ointment Formulation from Collagen Extract of Tilapia Fish Skin (*Oreochromis niloticus*) for Healing Burns in *Mus musculus. Pharmaceutical Journal of Indonesia.* 8(1): 9-15. https://doi.org/10.21776/ub.pji.2022.008.01.2

Oslan. S. N. H.. Li. C. X.. Shapawi. R.. Mokhtar. R. A. M.. Noordin. W. N. M.. & Huda. N. (2022). Extraction and Characterization of Bioactive Fish By-Product Collagen as Promising for Potential Wound Healing Agent in Pharmaceutical Applications: Current Trend and Future Perspective. *Internasional Journal of Food Science.* 2022(2): 1-10. https://doi.org/10.1155/2022/9437878

Paudi. R.. Sulistijowati. R.. & Mile. L. (2020). Rendemen Kolagen Kulit Ikan Bandeng (*Chanos chanos*) Segar Hasil Ekstraksi Asam Asetat. *Jambura Fish Processing Journal.* 2(1): 21-27. https://doi.org/10.37905/nj.v10i2.21036

Pei. M. (2020). Biocompatibility of synthetic dressings. *Journal of Biomaterials Applications.* 34(3): 439-451.

Plum. M.. Beier. J. P.. & Ruhi. T. (2025) Delayed Cutaneous Wound Healing in Young and Old Female Mice Is Associated with Differential Growth Factor Release but not Inflammatory Cytokine Secretion. *Biogerontology.* 16(1): 37-50. https://doi.org/10.1007/s10522-024-10179-7

Ramdhani. G.. & Ariani. A. (2016). *Pengambilan Kolagen Pada Sisik Ikan Dari Limbah Pabrik Fillet Ikan Menggunakan Metode Ekstraksi Asam* (Skripsi). Institut Teknologi Sepuluh Nopember.

Rodrigues. M.. Kosaric. N.. Bonham. C. A.. & Gurtner. G. C. (2019). Wound healing: A cellular perspective. Physiological Reviews. 99(1): 665-706. https://doi.org/10.1152/physrev.00067.2017

Romadhon. Darmanto. Y. S.. & Kurniasih. R. A. (2019). Karakteristik Kolagen dari Tulang. Kulit. dan Sisik Ikan Nila. *Jurnal Pengelolahan Hasil Perikanan Indonesia.* 22(2): 403-410. https://ejournal.unsrat.ac.id/index.php/JPKT/index

Safithri. M.. Tarman. K.. Suptijah. P.. & Widowati. N. (2019). Karakteristik fisikokimia kolagen larut asam dari kulit ikan parang-parang (Chirocentrus dorab). Jurnal Pengolahan Hasil Perikanan Indonesia. 22(3): 441-452. https://doi.org/10.17844/jphpi.v22i3.28924

Tambun. R.. Limbong. H. P.. Pinem. C.. & Manurung. E. (2016). Pengaruh ukuran partikel. waktu dan suhu pada ekstraksi fenol dari lengkuas merah. Jurnal Teknik Kimia USU. 5(4): 53-56.

Susanto. Y.. Solehah. F. A.. Fadya. A.. & Khaerani. K. (2023). Potensi Kombinasi Ekstrak Rimpang Kunyit (*Curcuma longa* L.) dan Kapur Sirih Sebagai Anti Inflamasi dan Penyembuhan Luka Sayat. *Journal of Pharmaceutical Science and Clinical Research.* 8(1): 32-45. DOI: 10.20961/jpscr.v8i1.60314

Utami. J. P.. Wasiaturrahmah. Y.. Carabelly. A. N.. Aspriyanto. D.. Nahzi. Y. I.. Arfin. R.. Ramadhaniyah. N.. & Jatmiko. H. N. (2025). Investigasi Farmakologis Aktivitas Penyembuhan Luka Mulut Gel Ekstrak Akar *Stachytarpheta jamaicensis* pada Tikus Wistar. *Jurnal Bioteknologi & Biosains Indonesia.* 12(1): 85-95. https://doi.org/10.55981/jbbi.2025.9390

Wahid. H.. Karim. S. F.. & Sari. N. (2022). Formulasi Sediaan Krim *Anti-aging* dari Ekstrak Kolagen Limbah Sisik Ikan Bandeng (*Chanos chanos*). *Jurnal Sains dan Kesehatan*. 4(4): 428-436. DOI:10.25026/jsk.v4i4.1289

Wahyuni. W.. Wahid. H.. & Febriani. R. (2022). Formulasi Krim Ekstrak Etanol Tangkai Daun Talas (*Colocasia esculanta* L.) Terhadap Luka Sayat Pada Tikus Putih (*Rattus norvegicus*) Galur Wistar. *Jurnal Kesehatan Tambusai*. 3(3): 338-347. DOI:10.31004/jkt.v3i3.5721

Wati. M. (2024). *Nanopartikel Perak (AgNPs) yang Disintesis dari Ekstrak Umbi Sarang Semut (Myrmecodia sp. Jack) sebagai Bahan Salep Untuk Penutupan Luka Sayat Pada Mencit* (Skripsi). Universitas Mulawarman.

Yampolsky. M.. Bachelet. I.. & Fuchs. Y. (2024). Reproducible Strategy for Excisional Skin Wound Healing Studies in Mice. *Nature protocol*. 19(1): 184-206. https://doi.org/10.1038/s41596-023-00899-4

Zaelani. B.. Safitri. M.. Tarman. K.. Setyaningsih. I.. & Meyda. (2019). Collagen Isolation with Acid Soluble Method from the Skin of Red Snapper (*lutjanus* sp.). *IOP Conference series: Earth and Enviromental Science*. 241(2019): 1-9. DOI: 10.1088/1755-1315/241/1/012033