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Research Paper

Biopotential of Gorontalo Hulu'u Fish (*Giuris margaritacea*) Albumin in a Novel Spray Gel Formulation for the Treatment of Burn Wounds: *In Vivo* Evaluation in Rats

Mohamad Aprianto Paneo[1*], Endah Nurrohwinta Djuwarno[1], Sitty Ainsyah Habibie[2], Nurain Thomas[1], Multiani S. Latif[1], Nur Alifia Karina Munafri[1], Rayhan Firman Anasiru[1]

[1]*Department of Pharmacy, Faculty of Sport and Health, Universitas Negeri Gorontalo, Gorontalo, 96128, Indonesia*
[2]*Department of Aquatic Resources Management Faculty of Marine Affairs and Fisheries Technology, Universitas Negeri Gorontalo, Gorontalo, 96128, Indonesia*
***Corresponding author**: apriyanto07@ung.ac.id*

Abstract
This study aimed to evaluate the efficacy of different concentrations of Hulu'u fish albumin-based spray gels in promoting wound healing in burn injuries in male white rats. The formulations included 10%, 15%, and 20% concentrations of Hulu'u fish albumin, which were compared to a commercial snakehead fish albumin gel (positive control) and a spray gel base without albumin (negative control). Wound diameter reduction was assessed over seven days. The results indicated a clear dose response relationship, with the 20% Hulu'u fish albumin formulation (F3) achieving the most significant reduction in wound diameter, averaging 1.57 mm (range: 1.5–1.6 mm), representing a 91.8% improvement compared to the negative control group, which showed an average reduction of 19.13 mm (range: 18.1–19.7 mm). The Positive Control (snakehead fish albumin gel) demonstrated a moderate reduction with an average of 6.97 mm (range: 6.6–7.6 mm). The 10% Hulu'u fish albumin (F1) and 15% Hulu'u fish albumin (F2) formulations showed moderate improvements, with average reductions of 4.73 mm (range: 4.4–5.1 mm) and 4.5 mm (range: 4.7–3.9 mm), respectively. These findings suggest that higher concentrations of Hulu'u fish albumin, particularly the 20% formulation, offer superior wound healing properties, outperforming both the negative control and the commercial snakehead fish albumin gel. The study highlights the potential of Hulu'u fish albumin as a novel bioactive compound for burn wound treatment and warrants further investigation for clinical applications.

Keywords
Hulu'u Fish Albumin, Spray Gel, Burn Wounds, Wound Healing, Dose-Response, Rat Model

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1. INTRODUCTION

Burn wounds remain a major clinical problem because they are associated with high risk of infection, prolonged inflammation, delayed re-epithelialization, and potential scarring. Effective topical therapies are therefore required to accelerate tissue repair while maintaining a moist wound environment and minimizing contamination. In this context, bioactive-based wound dressings and hydrogels have been increasingly explored as practical approaches to improve burn wound outcomes (Al-Romaima et al., 2022; Rodrigues et al., 2023; Wang et al., 2022).

Fish derived albumin has gained attention as a promising bioactive component for wound care due to its physicochemical and biological properties, including high solubility, antioxidant capacity, anti-inflammatory effects, and its role in maintaining osmotic balance and supporting cellular regeneration. Compared with conventional mammalian albumin, albumin from marine and freshwater sources may offer advantages such as lower allergenicity and the presence of bioactive peptides generated during enzymatic degradation. These characteristics support the use of fish albumin as an innovative ingredient in topical formulations intended to promote wound healing (Giannetto et al., 2020; Suzuki et al., 2020). Hulu'u fish (*Giuris margaritacea*), a species native to Gorontalo, Indonesia, represents a locally abundant but underexplored source of albumin for biomedical applications. Preliminary evidence suggests that Hulu'u fish albumin may exhibit favorable biochemical characteristics, including an appropriate molecular-weight profile, high protein purity, and potential regenerative or anti-inflammatory effects. However, despite its regional availability and cultural relevance, scientific studies focusing on its application in wound management remain limited. This gap highlights an opportunity to translate local biodiversity into accessible and innovative therapeutic products, particularly for topical

wound care (Jusuf et al., 2025; Mahdipour and Mequanint, 2022). From a pharmaceutical delivery perspective, a sprayable gel system offers practical advantages for burn wounds by enabling uniform application over irregular surfaces, supporting controlled coverage, maintaining hydration, and reducing handling-related contamination. Spray gel formulations also facilitate convenient administration and may improve patient compliance compared with conventional semisolid products. Incorporating albumin into a sprayable gel matrix is therefore a strategic approach to maximize the biological function of albumin while ensuring a feasible topical dosage form (Meng et al., 2023; Paula et al., 2022; Pomalango et al., 2025; Thomas et al., 2025). Accordingly, this study aimed to develop Hulu'u fish albumin-based spray gel formulations and evaluate their therapeutic performance in an experimental burn wound model in rats. The work included physicochemical characterization and *in vivo* assessment of wound healing to provide foundational evidence supporting the utilization of Hulu'u fish albumin as a novel local bioresource for modern burn wound management (Jahanabadi et al., 2015; Lee and Lin, 2022; Sathyaraj et al., 2023).

2. EXPERIMENTAL SECTION

2.1 Material

Fresh Hulu'u fish (*Giuris margaritacea*) meat was obtained from Lake Limboto, Gorontalo, Indonesia. The edible muscle tissue was used as the source of albumin. N-hexane (Emsure®), Propylene glycol (Supelco®), Hydroxypropyl methylcellulose (HPMC) (Green Excipient®), Poloxamer 188 (Corning®), Glycerin (Green Excipient®), DMDM hydantoin (Green Excipient®), and distilled water (aquadest) (Green Excipient®) were used as excipients for the spray gel formulation. All chemicals and solvents were of analytical grade and used as received.

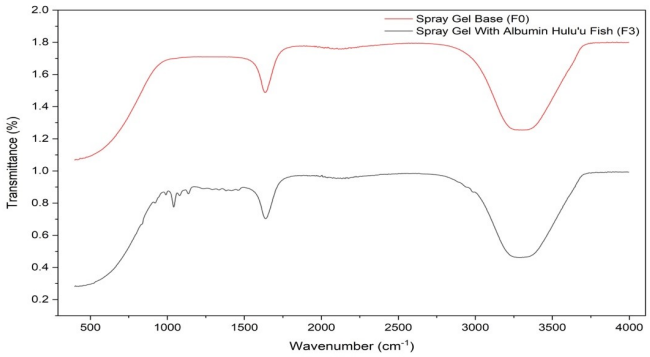


Figure 1. Overlay Fourier Transform Infrared (FTIR) Spectra of Spray Gel Base (F0) and Albumin-Loaded Spray Gel (F3)

2.2 Instruments

UV–Vis spectrophotometry (Thermolab®), Differential scanning calorimetry (DSC) (Thermolab®), Fourier transform infrared (FTIR) (Bruker®), Climatic chamber (Thermolab®), Franz Diffusion Cells (Copley®), and Centrifuge (Joanlab MC-12 Pro®).

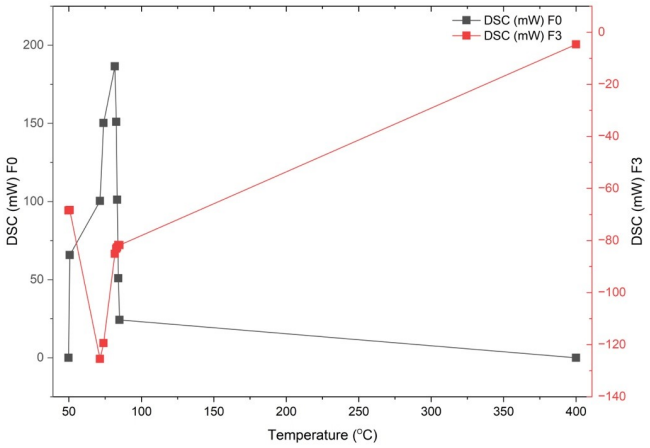


Figure 2. Differential Scanning Calorimetry (DSC) Analysis. (Black Line) Spray Gel without Hulu'u Fish (*Giuris margaritacea*) Albumin. (Red Line) Spray gel (F3) Hulu'u Fish (*Giuris margaritacea*) Albumin

2.3 Extraction Hulu'u Fish (*Giuris margaritacea*) Albumin

Fresh Hulu'u fish (*Giuris margaritacea*) were cleaned by removing the viscera, scales, and gills, followed by thorough washing with water to minimize microbial contamination, as these tissues harbor the highest microbial load. The fish were then filleted, the skin discarded, and the muscle tissue collected as the albumin source. A total of 100 g of muscle was weighed, cut into small pieces, and homogenized using a blender to increase surface area and facilitate extraction. Albumin extraction was performed using a thermal assisted method in a water bath: the homogenized muscle was placed into a 500 mL beaker containing 100 mL of distilled water and incubated at 56 °C for 10 minutes. After heating, the mixture was filtered through clean muslin cloth to separate the filtrate and residue, and the residue was weighed to determine extraction yield. The filtrate volume was measured and transferred into a separatory funnel, then mixed with n-hexane at a ratio of 1:4 (v/v) to remove lipids. The mixture was homogenized for 30 minutes and allowed to stand until two phases formed, with an upper n-hexane layer containing dissolved lipids and a lower aqueous layer containing the albumin extract. The aqueous phase was collected and centrifuged at 4,500 rpm for 30 minutes to remove remaining particulate matter. The resulting supernatant was considered the purified Hulu'u fish albumin extract and was used for subsequent spray gel formulation (Hospice et al., 2018).

2.4 Biuret Test for Preliminary Protein Verification

A qualitative Biuret test was performed as a preliminary assay to verify the presence of protein in the obtained Hulu'u fish

Table 1. Formula Spray Gel Hulu'u Fish (*Giuris margaritacea*) Albumin

Component	Formula (%)				Function
	F1	F2	F3	F4	
Hulu'u Fish (*Giuris margaritacea*) Albumin	10	15	20	0	Active Pharmaceutical Ingredient
Hydroxypropyl methylcellulose (HPMC)	0.5	0.5	0.5	0.5	Gelling Agent
Poloxamer 188	2	2	2	2	Gelling Agent
Propylene glycol	15	15	15	15	Solvent
Glycerin	5	5	5	5	Moisturizer
DMDM hydantoin	0.6	0.6	0.6	0.6	Preservative
Aquadest	Ad 100	Ad 100	Ad 100	Ad 100	Solvent

Note : F4 : Negative Control

Table 2. Animal Treatment Formulation

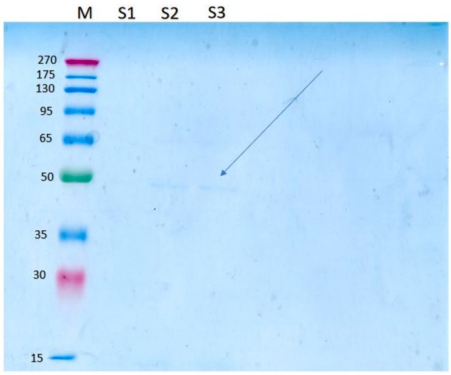
Group	Treatment formulation	Description
1	Spray gel base (without albumin)	Negative control: rats treated with spray gel base only, without Hulu'u fish albumin.
2	10% Hulu'u fish albumin spray gel	Rats treated topically with spray gel containing 10% Gorontalo Hulu'u fish albumin.
3	15% Hulu'u fish albumin spray gel	Rats treated topically with spray gel containing 15% Gorontalo Hulu'u fish albumin.
4	20% Hulu'u fish albumin spray gel	Rats treated topically with spray gel containing 20% Gorontalo Hulu'u fish albumin.
5	Commercial snakehead fish albumin gel	Positive control: rats treated with a marketed gel containing snakehead (*Channa striata*) albumin.

albumin extract. A commercially available Biuret reagent was used for the analysis. Three types of solutions were prepared in separate test tubes: a blank containing distilled water, a positive control containing a standard albumin solution, and the sample containing the Hulu'u fish albumin extract. For each tube, 1 mL of solution was mixed with 4 mL of Biuret reagent, vortexed gently, and incubated at room temperature for 20–30 minutes. The development of a violet to purple coloration in the sample and positive control tubes, compared with the blank, was interpreted as a positive result indicating the presence of protein in the albumin extract. In addition to visual inspection, absorbance could be measured spectrophotometrically at approximately 540–550 nm against the blank to support qualitative observation (Subroto et al., 2020).

2.5 Protein Characterization by SDS-PAGE

SDS-PAGE was used to characterize the protein profile of Hulu'u fish albumin in spray gel formulations. The samples were prepared with SDS, reducing agents, and glycerol, then denatured at 95 °C. A 12% polyacrylamide gel was used for electrophoresis at 120V for 90 minutes. After staining with Coomassie Brilliant Blue and de staining, the gel was analyzed to determine the albumin's molecular weight and concentration. Densitometric analysis, using ImageJ software, quantified the albumin by comparing protein band intensities with a molecular weight marker. This analysis confirmed protein integrity and concentration, ensuring the therapeutic efficacy and stability of the albumin within the spray gel formulation (Qureshi and Tahniyath, 2025).

Table 3. SDS-Page Test Protein Verification

Sample	Results SDS-Page
Marker Board Color Prestained Protein Standard GenScrips. Hulu'u fish albumin extract With 10% Concentration. Protein Ladder GenScript Stained Protein : Cat. No : M00624-250 Lot. No : M2205016 Description : M : Marker S : Sample (with 3 Replication) Note : readable level at ~48 kDa	

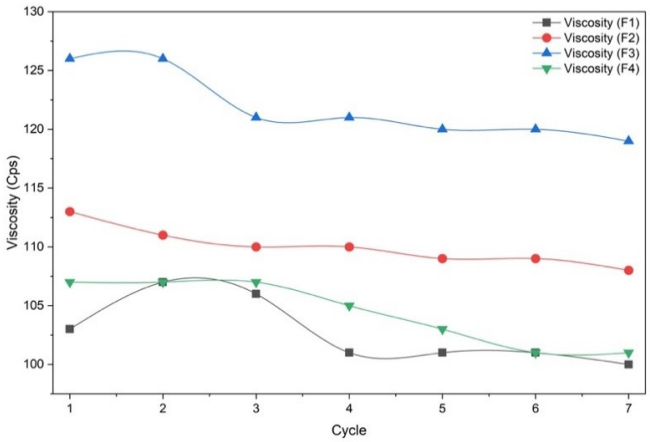


Figure 3. Viscosity Profile of Spray Gel

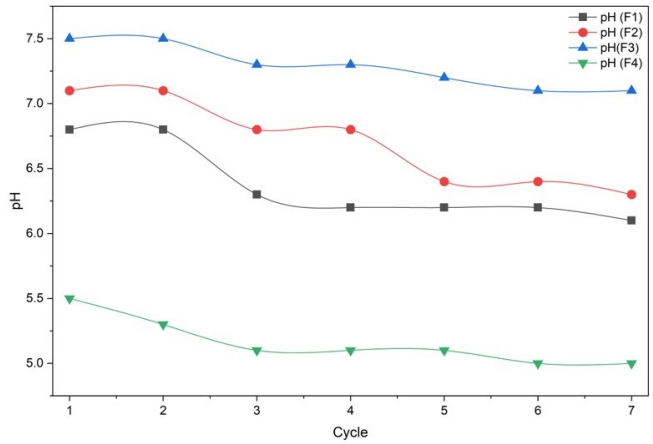


Figure 4. pH Profile of Spray Gel

2.6 Fourier Transform Infrared (FTIR) Spectroscopic Analysis

Fourier transform infrared (FTIR) spectroscopy was performed to identify the functional groups present in the Hulu'u fish albumin extract and to evaluate possible interactions between albumin and excipients within the spray gel formulations. Samples of pure albumin extract, excipients, and the final spray gel were prepared in dried form and analyzed using an FTIR spectrophotometer operated in the range of 4000–400 cm^{-1}. Each spectrum was recorded at room temperature with an appropriate resolution to ensure accurate detection of characteristic absorption peaks. The resulting spectra were compared to assess shifts, peak intensities, or the emergence of new bands, which may indicate chemical interactions, structural modifications, or successful incorporation of albumin into the gel matrix (Alhazmi et al., 2021; Mahdipour and Mequanint, 2022).

2.7 Differential Scanning Calorimetry (DSC) Analysis

Differential scanning calorimetry (DSC) analysis was conducted to evaluate the thermal behavior and potential shifts in stability of the Hulu'u fish albumin within the spray gel formulations. Samples of pure albumin extract, individual excipients, and the final spray gel preparations were weighed accurately and placed in aluminum pans, which were then hermetically sealed. The DSC instrument was operated under a nitrogen atmosphere, and samples were scanned over an appropriate temperature range to detect endothermic or exothermic transitions. Thermograms obtained from the albumin, excipients, and formulated gels were compared to assess changes in melting points, denaturation temperatures, or peak profiles that may indicate molecular interactions, structural modifications, or the stabilization of albumin within the gel matrix (Durowoju et al., 2017; Hawe and Friess, 2006; Jia and Zhang, 2014).

Table 4. FTIR Peak Assignments of Hulu'u Fish (*Giuris margaritacea*) Albumin, Spray Gel Base, and Albumin-Containing Spray Gel Formulations

Wavenumber (cm^{-1})	Band	Functional group	Main component (s)	Interpretation
3300	Amide A / O–H	N–H stretching (protein) and O–H stretching	Albumin, HPMC, glycerin	Overlapping protein N–H and polymer/humectant O–H groups
2900	C–H stretch	Asymmetric and symmetric C–H stretching ($–CH_2–$, $–CH_3$)	HPMC, poloxamer 188, glycerin	Alkyl chains of polymeric and surfactant backbones
1650	Amide I	C=O stretching of peptide bonds	Hulu'u fish albumin	Indicative of protein secondary structure (amide I band)
1540	Amide II	N–H bending and C–N stretching	Hulu'u fish albumin	Confirms presence of peptide bonds (amide II band)
1050–1150	C–O / C–O–C	C–O stretching and ether vibrations	HPMC, poloxamer 188, glycerin	Characteristic of polysaccharide and nonionic surfactant matrix
< 900	Fingerprint	Complex bending and skeletal vibrations	Entire formulation	Overall structural fingerprint of the spray gel

2.8 Formulation Spray Gel Hulu'u Fish (*Giuris margaritacea*) Albumin

The spray gel formulations were prepared by incorporating Hulu'u fish (*Giuris margaritacea*) albumin at concentrations of 10%, 15%, and 20% (w/w) in formulations F1, F2, and F3, respectively, while formulation F4 served as a blank gel base with no albumin. Hydroxypropyl methylcellulose (HPMC) at 0.5% (w/w) and poloxamer 188 at 2% (w/w) were utilized as gelling agents to provide the desired viscosity and sprayability of the gel. Poloxamer 188 and HPMC were added drop by drop to the formulation to ensure the consistency of the gel, preventing any formation of clumps and ensuring uniform dispersion throughout the mixture. Propylene glycol, at 15% (w/w), acted as both a solvent and co-solvent, while glycerin, at 5% (w/w), served as a moisturizer and humectant. DMDM hydantoin, at 0.6% (w/w), was incorporated as a preservative. Distilled water was added to each formulation to bring the total volume to 100%, with the concentration of excipients remaining constant across all formulations, differing only in the level of albumin. The process began by preparing the gelling matrix by thoroughly mixing Hydroxypropyl methylcellulose and poloxamer 188 drop by drop to form the gel base. Propylene glycol and glycerin were then added to the mixture, followed by DMDM hydantoin as the preservative. The albumin extract was incorporated into the gel at the specified concentrations for F1, F2, and F3, with no albumin added to F4. After ensuring uniform dispersion, distilled water was added to each formulation to complete the mixture, maintaining the overall composition of excipients. The resulting formulations were then subjected to physical evaluations, including viscosity, pH, and drying time, to confirm the suitability of the gel for spray application. Stability testing was conducted under controlled temperature and humidity conditions to assess the long-term physical and chemical stability of the spray gels (Puspitasari and Suprayitno, 2020). The dosage form formula can be seen in Table 1 below.

2.9 Evaluation Physical Characteristics of the Hulu'u Fish Albumin Spray Gel

The physical characteristics of the Hulu'u fish albumin spray gel formulations were evaluated through organoleptic, viscosity, pH, and drying-time assessments, with stability testing performed under controlled temperature and humidity conditions in a climatic chamber. Organoleptic examination was conducted by observing the color, odor, and appearance of each formulation under standard laboratory lighting conditions. Viscosity was measured using a rotational viscometer at ambient temperature to determine the flow behavior and consistency

Table 5. Physical Stability Testing of Spray Gel

Cycle	Parameters	Results			
		F1	F2	F3	F4
1	Viscosity (Cps)	103	113	126	107
	pH	6.8	7.1	7.5	5.5
2	Viscosity (Cps)	107	111	126	107
	pH	6.8	7.1	7.5	5.3
3	Viscosity (Cps)	106	110	121	107
	pH	6.3	6.8	7.3	5.1
4	Viscosity (Cps)	101	110	121	105
	pH	6.2	6.8	7.3	5.1
5	Viscosity (Cps)	101	109	120	103
	pH	6.2	6.4	7.2	5.1
6	Viscosity (Cps)	100	109	120	101
	pH	6.2	6.4	7.1	5.0
7	Viscosity (Cps)	100	108	119	101
	pH	6.1	6.3	7.1	5.0
SD (Deviation Standard) Viscosity		± 2.88	± 1.63	± 2.91	± 2.76
SD (Deviation Standard) pH		± 0.23	± 0.34	± 0.17	± 0.18

Note : F4 : Negative Control

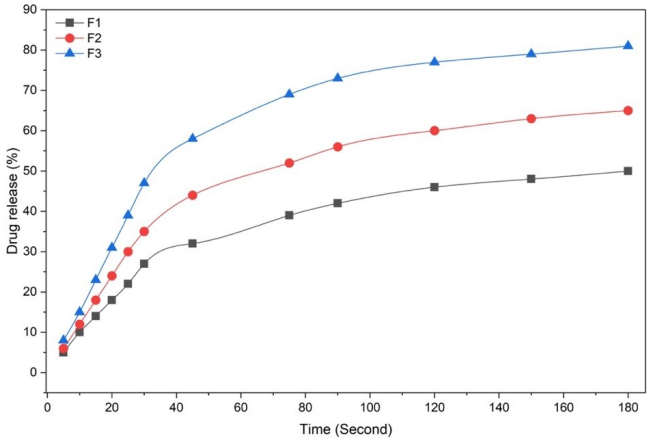


Figure 5. In Vitro Cumulative Release Profiles of Hulu'u Fish Albumin from Spray Gel Formulations (F1–F3)

of the gels. The pH of each formulation was assessed using a calibrated digital pH meter to ensure compatibility with skin application. Drying-time testing was performed by applying a fixed amount of spray gel onto a glass surface and recording the time required for the formulation to form a dry film. For stability evaluation, the formulations were subjected to seven cycles of temperature stress in a climatic chamber, each cycle consisting of storage at 4 °C for 24 hours followed by 40 °C for 24 hours. These evaluations were carried out to verify the physical stability and usability of the developed spray gel preparations (Della Sala et al., 2023; Lal et al., 2023).

2.10 *In Vitro* Penetration Study Using Franz Diffusion Cells

Penetration studies of the Hulu'u fish albumin spray gel were performed using a Franz diffusion cell system to evaluate the permeation of albumin across the membrane. Each formulation was applied to the donor compartment, while the receptor compartment was filled with phosphate-buffered solution maintained at 37 ± 0.5 °C and continuously stirred to ensure homogeneity. A synthetic membrane suitable for protein diffusion studies was mounted between the donor and receptor chambers. At predetermined time intervals, aliquots were withdrawn from the receptor compartment and immediately replaced with an equal volume of fresh buffer to maintain sink conditions. The concentration of albumin permeating through the membrane was quantified using a UV–Vis spectrophotometer at the appropriate absorption wavelength, and penetration profiles were constructed to assess the release and diffusion behavior of the albumin from the spray gel formulations (Iliopoulos et al.,

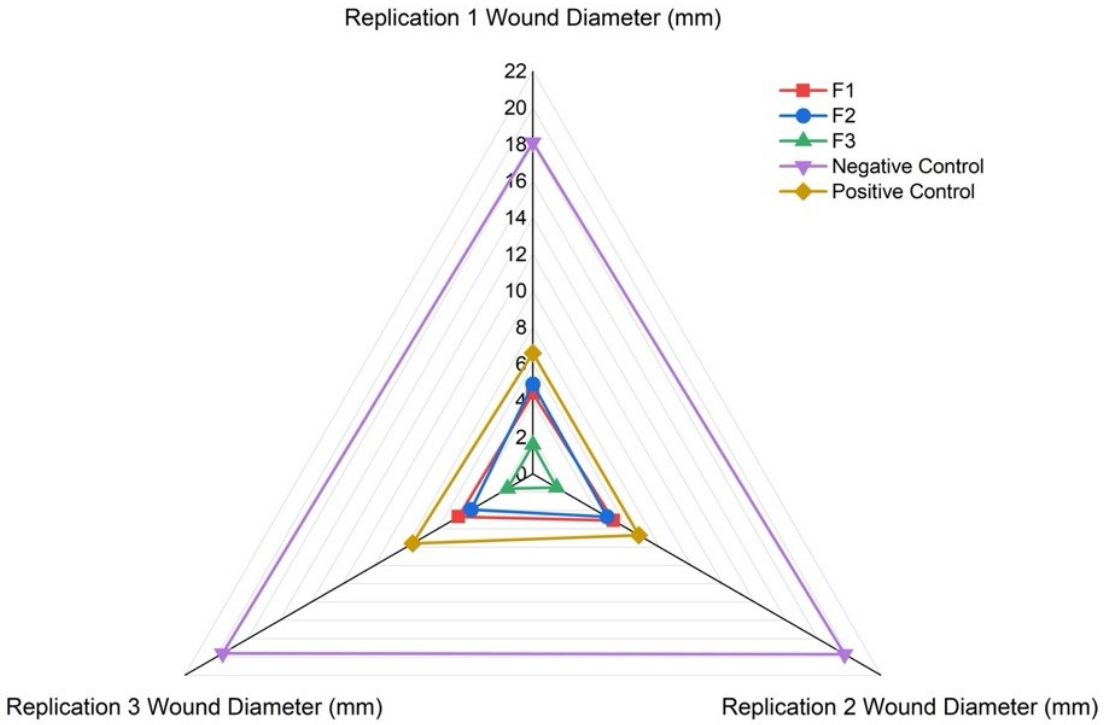


Figure 6. Graph of Wound Diameter (Average) Reduction in Burn-Injured Rats (Treatment Effects) Over 7 Days

Table 6. Descriptive Analysis of Viscosity Spray gel

Formula	N (total)	Mean (Cps)	Std. Deviation	Minimum (Cps)	Maximum (Cps)
1	7	102.57	2.88	100	107
2	7	110.00	1.63	108	113
3	7	121.86	2.91	119	126
4	7	104.43	2.76	101	107

2020; Pulsoni et al., 2022).

2.11 Animals and Ethical Consideration

A total of male white rats were used in this study and randomly allocated into five experimental groups. All animals were acclimatized under controlled environmental conditions for a minimum of seven days prior to experimentation, following institutional ethical procedures. The experimental groups were arranged as follows: Group 1 (negative control) received only the spray gel base without Hulu'u fish albumin; Group 2 received spray gel containing 10% Hulu'u fish albumin; Group 3 received spray gel containing 15% Hulu'u fish albumin; Group 4 received spray gel containing 20% Hulu'u fish albumin; and Group 5 (positive control) received a commercially available gel containing snakehead fish (*Channa striata*) albumin, which is routinely used for burn wound treatment. All procedures involving animals were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals (De Souza et al., 2024). Ethical approval for the use of rats in this research was obtained from the Health Research Ethics Committee (KEPK), Universitas Negeri Gorontalo, under approval number 185A/UN47.B7/KE/2025. As researchers, we have ensured that all animal treatments were conducted in full accordance with ethical guidelines for animal experimentation, prioritizing their welfare throughout the study, as detailed in Table 2.

2.12 Burn Wound Induction and Treatment Protocol

Burn wounds were created on male white rats assigned into five experimental groups: F1 (10% albumin spray gel), F2 (15% albumin spray gel), F3 (20% albumin spray gel), negative control (spray gel base without albumin), and positive control (commercial snakehead fish albumin gel). Prior to wound induction, all animals were anesthetized using topical lidocaine gel sup-

Table 7. Analysis of Variance (ANOVA) One Way of Viscosity

Viscosity	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1585.429	3	528.476	78.155	<.001
Within Groups	162.286	24	6.762		
Total	1747.714	27			

Table 8. Descriptive Analysis of pH Spray gel

Formula	N (total)	Mean (Cps)	Std. Deviation	Minimum (Cps)	Maximum (Cps)
1	7	6.371	0.23	6.1	6.8
2	7	6.700	0.34	6.3	7.1
3	7	7.286	0.17	7.1	7.5
4	7	5.157	0.18	5.0	5.5

plemented with an analgesic agent to minimize procedural pain and distress. The dorsal area of each rat was shaved and disinfected, after which a standardized circular burn wound measuring 2 cm in diameter was produced using a heated metal template applied with consistent pressure and contact time. Immediately following wound creation, each animal received the designated formulation according to its group assignment. All procedures were conducted in accordance with ethical guidelines for animal experimentation, ensuring adequate analgesia, monitoring, and humane handling throughout the study (Cai et al., 2014; Utariani et al., 2020).

2.13 Data Analysis

For the statistical analysis, descriptive statistics were calculated using SPSS 30 to summarize the central tendency and variability of key variables, including spray distance, spray diameter, and weight. This involved computing the mean, standard deviation, minimum, and maximum values for each parameter across the different concentrations of Hulu'u fish albumin (10%, 15%, and 20%). To assess significant differences between the groups, a one-way analysis of variance (ANOVA) was conducted. This analysis allowed us to determine the effects of albumin concentration on the spray gel characteristics and evaluate the statistical significance of these effects (Prinn et al., 2002; Kim, 2017; Assaad et al., 2014).

3. RESULT AND DISCUSSIONS

The albumin extract appears as a clear-to-slightly turbid aqueous solution without visible particulates, suggesting that the thermal-assisted extraction and defatting step did not induce gross protein coagulation. For the formulations (F1–F3), the absence of visible phase separation, precipitation, or sedimentation across increasing albumin concentrations indicates adequate compatibility between the protein and the gel matrix (HPMC–poloxamer system). This visual homogeneity supports the formulation objective of producing a sprayable gel that maintains uniform distribution of the bioactive protein prior to subsequent physicochemical (FTIR/DSC) and biological (*in vivo*) evaluations (Mahdipour and Mequanint, 2022; Meng et al., 2023; Ong et al., 2020). The violet/purple coloration (relative to the blank) indicates the presence of proteins/peptides, confirming that the extraction process successfully recovered proteinaceous material. This test is intended as an initial screening step; therefore, the identity and integrity of albumin were subsequently confirmed using SDS-PAGE (molecular weight profile) and supported by FTIR/DSC analyses in the formulated product (Kuprina et al., 2023; Mahdipour and Mequanint, 2022). The presence of the 48 kDa band confirms the presence of Hulu'u fish albumin, which serves as the bioactive protein for wound healing applications. The bands in samples S1, S2, and S3, representing three replicates of the 10% albumin formulation, show consistent results, indicating reliable extraction and successful incorporation of albumin into the gel. The intensity and position of the band suggest that the albumin is highly pure, with minimal degradation during the extraction and formulation processes. No additional bands or significant degradation products are observed, supporting the integrity of the albumin in the spray gel formulation. Moreover, the consistent protein profile across replicates (S1, S2, S3) demonstrates the reproducibility of the preparation method. This analysis confirms that the extracted Hulu'u fish albumin is of sufficient quality and purity for incorporation into the spray gel formulation. The protein band at approximately 48 kDa corresponds to fish derived albumin, validating the extraction protocol's effectiveness and ensuring the protein's stability within the gel matrix. Thus, this SDS-PAGE analysis not only validates the presence of Hulu'u fish albumin in the extract but also confirms that the extraction process was carried out effectively, maintaining the protein's integrity for its intended therapeutic use in burn wound healing (Sulistiawati et al., 2025). The results of protein verification using SDS-PAGE can be seen in Table 3.

Table 9. Analysis of Variance (ANOVA) One Way of pH

pH	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	16.927	3	5.642	85.707	<.001
Within Groups	1.580	24	0.066		
Total	18.507	27			

Table 10. Characteristics Spray Distance, Spray Diameter, Spreadability and Adhesion Spray Gel

Concentration	Spray Distance (cm)	Spray Diameter (mm)	Weight (mg)	Spreadability	Adhesion
10%	3	7.7	106.1	Droplet	Adherent
		7.4	106		
		7.6	105.8		
	Mean	7.6	105.9		
	Std. Dev ±	0.129	0.125		
	5	11.3	100.3	Droplet	Adherent
		11.8	100.7		
		11.6	100.2		
	Mean	11.5	100.4		
	Std. Dev ±	0.205	0.216		
15%	3	8.2	131	Droplet	Adherent
		8.5	131.5		
		8.7	130.8		
	Mean	8.4	131.1		
	Std. Dev ±	0.205	0.294		
	5	10.8	115.4	Droplet	Adherent
		10.3	115.1		
		10.9	115.7		
	Mean	10.6	115.4		
	Std. Dev ±	0.263	0.245		
20%	3	10.4	118.1	Droplet	Adherent
		10.3	118		
		10.7	118.3		
	Mean	10.4	118.1		
	Std. Dev ±	0.170	0.125		
	5	12.4	140.3	Droplet	Adherent
		12.1	140.1		
		12.3	140.9		
	Mean	12.2	140.3		
	Std. Dev ±	0.125	0.340		

The spectrum of F3 shows characteristic protein-related bands, including amide A (~3300 cm^{-1}), amide I (~1650 cm^{-1}), and amide II (~1540 cm^{-1}), which are absent or less pronounced in the base formulation. The preservation of major polymer related bands and the absence of new peaks indicate successful incorporation of Hulu'u fish albumin without chemical degradation. FTIR spectra of the spray gel base (F0) and albumin loaded formulation (F3) were replotted from raw instrumental data to improve clarity and consistency (Figure 1). The F3 spectrum exhibited distinct protein-related absorption bands, including amide A (~3300 cm^{-1}), amide I (~1650 cm^{-1}), and amide II (~1540 cm^{-1}), confirming the presence of

Table 11. *In vitro* Cumulative Release (%) of Hulu'u Fish Albumin from Spray Gel Formulations (F1–F3) Using Franz Diffusion Cells

Time (min)	F1 (%)	F2 (%)	F3 (%)
5	5	6	8
10	10	12	15
15	14	18	23
20	18	24	31
25	22	30	39
30	27	35	47
45	32	44	58
75	39	52	69
90	42	56	73
120	46	60	77
150	48	63	79
180	50	65	81
Average	29.42	38.75	50

peptide bonds associated with albumin (Salamah et al., 2025). The overall similarity of the polymer-related bands between F0 and F3 suggests that albumin incorporation did not alter the chemical integrity of the gel matrix. Minor shifts in the amide region likely reflect non covalent interactions, such as hydrogen bonding, between albumin and the hydrophilic polymers (Hassan et al., 2021). The FTIR test results can be seen in Figure 1, and the wavelength and transmittance can be seen in Table 4.

Overlay analysis of the base and albumin-containing spectra demonstrated that the main functional groups of both the polymeric matrix and albumin remained intact in the final product. Slight shifts in the amide I and amide II regions suggest non-covalent interactions between albumin and hydrophilic domains of HPMC and poloxamer 188, most likely through hydrogen bonding. Such interactions are desirable because they can enhance physical stabilization of the protein within the gel network without indicating chemical degradation, as no new peaks or disappearance of key bands were observed. Overall, the FTIR data support the compatibility of Hulu'u fish albumin with the spray gel excipients and indicate that the formulation process maintains the structural features required for its intended biological activity in burn wound applications (Chander et al., 2021; Meng et al., 2023).

The DSC analysis reveals distinct thermal characteristics between the spray gel without Hulu'u fish (*Giuris margaritacea*) albumin (F0) and the spray gel with albumin (F3). Both samples exhibit an endothermic peak around 100-110 °C, indicating water evaporation or phase transitions, with the F3 gel showing a lower intensity, suggesting that the presence of albumin may reduce the evaporation rate and enhance thermal stability. Additionally, the F3 sample exhibits an exothermic peak between 190-210 °C, absent in the F0 gel, which may be attributed to the protein's structural changes or dehydration at higher temperatures, indicating that albumin plays a stabilizing role in the formulation. These findings demonstrate that albumin from Hulu'u fish contributes to the improved thermal stability of the spray gel, offering potential benefits for pharmaceutical or cosmetic applications, particularly in products requiring enhanced thermal resistance. Further studies are recommended to explore the interactions between albumin and other components within the spray gel formulation (Durowoju et al., 2017; Ibarra-Molero et al., 2011; Seelig and Schönfeld, 2016). The results of the DSC analysis can be seen in Figure 2.

The viscosity profiles of the four spray gel formulations (F1–F4) across seven stability cycles are shown in Figure 3. Overall, all formulations demonstrated minimal fluctuations in viscosity during the alternating temperature stress conditions (4 °C/40 °C, 24 h each cycle). F3, which contained the highest concentration of Hulu'u fish albumin, consistently exhibited the highest viscosity values (126–119 cPs), indicating a more structured gel matrix likely due to increased protein–polymer interactions. F1 and F2 showed moderate viscosity levels with minor declines over time, whereas the negative control (F4) maintained the lowest viscosity values (107–101 cPs). The low standard deviation values (SD ± 2.88 for F1, ± 1.63 for F2, ± 2.91 for F3, and ± 2.76 for F4) confirm that all formulations maintained rheological stability throughout the cycling test (Table 5). These findings indicate that the incorporation of albumin did not induce destabilization or phase alteration of the gel network. The pH profiles throughout the seven stability cycles are presented in Figure 4. All formulations showed only slight decreases in pH, remaining within acceptable dermal pH ranges suitable for topical application. F3 maintained the highest pH values (7.5–7.1), consistent with the mild alkalinity of albumin containing formulations, while F4 displayed slightly acidic behavior (5.5–5.0), reflecting its excipient-only composition. The small standard deviation values (SD ± 0.23 for F1, ± 0.34 for F2, ± 0.17 for F3, and ± 0.18 for F4) indicate excellent pH stability under temperature cycling (Table 5). Importantly, no abrupt pH shifts were observed, suggesting the absence of protein degradation, excipient hydrolysis, or other chemical instabilities. Together, the viscosity and pH stability results confirm that all spray gel formulations, particularly those containing Hulu'u fish albumin, remain physically robust and suitable for further pharmaceutical evaluation (Adriano et al., 2014; Dantas et al., 2016; Hussein et al., 2022).

Table 6 presents the descriptive analysis of viscosity for each spray gel formulation tested. The results indicate that the formulation with the highest concentration of Hulu'u fish albumin (F3) exhibited the highest average viscosity at 121.86 cP, compared to formulations F1 and F2, which had average viscosities of 102.57 cP and 110.00 cP, respectively. This data shows a significant relationship between albumin concentration and gel viscosity, with higher concentrations of albumin resulting in thicker gels. In contrast, the negative control (F4)

Table 12. Descriptive Analysis *In vitro* Cumulative Release (%) of Hulu'u Fish Albumin from Spray Gel Formulations (F1–F3)

Formula	N (total)	Mean (%)	Std. Deviation	Minimum (%)	Maximum (%)
1	12	29.42	15.675	5	50
2	12	38.75	20.794	6	65
3	12	50.00	26.447	8	81

Table 13. Analysis of Variance (ANOVA) *In vitro* Cumulative Release (%) of Hulu'u Fish Albumin from Spray Gel Formulations (F1–F3)

Drug release (%)	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2549.389	2	1274.694	2.776	<.077
Within Groups	15153.167	33	459.187		
Total	17702.556	35			

had the lowest average viscosity at 104.43 cP, suggesting that a gel without albumin does not form as thick a consistency. This is further supported by the ANOVA results, which indicate significant differences between the groups ($p < 0.001$) as shown in Table 7. These findings confirm that Hulu'u fish albumin plays a critical role in achieving the desired viscosity for gel application in burn wounds (Table 7). Table 8 shows the pH analysis, revealing clear differences in pH values across the spray gel formulations. The 20% albumin formulation (F3) exhibited the highest pH at 7.29, while the negative control (F4) demonstrated the lowest pH at 5.16. This difference in pH indicates that albumin content can influence the gel's pH, which in turn affects its skin compatibility for topical use. The ANOVA results in Table 9 support this, showing significant differences between the groups ($p < 0.001$), further confirming the impact of albumin on the pH stability of the gel. Overall, these results suggest that higher concentrations of Hulu'u fish albumin not only increase gel viscosity but also influence its pH, which is essential for ensuring the gel's safety and effectiveness when applied to burn wounds (Tables 8 and 9).

The characteristics of the spray gel formulations, including spray distance, spray diameter, spreadability, and adhesion, were evaluated and summarized in Table 10. As the concentration of Hulu'u fish albumin increased, both the spray distance and spray diameter improved, with the 20% formulation showing the largest diameter (12.4 mm at 5 cm). The weight of the gel formulations also increased with higher albumin concentrations, with the 20% formulation weighing 118.1 mg at 3 cm, indicating enhanced gel formation and stability. All formulations demonstrated "droplet" spreadability and "adherent" adhesion, ensuring effective coverage and sustained contact with the wound surface. These findings suggest that higher albumin concentrations contribute to better sprayability, stability, and adhesion, making the formulations promising for effective burn wound treatment (Hájovská et al., 2020).

The *in vitro* penetration study demonstrated distinct release behaviors among the three spray gel formulations containing 10% (F1), 15% (F2), and 20% (F3) Hulu'u fish albumin. As shown in Figure 5 and Table 10, all formulations exhibited time-dependent increases in cumulative albumin release over 180 minutes, indicating effective diffusion of the active component through the synthetic membrane. However, the extent and rate of release varied substantially with increasing albumin concentration. F1 showed the lowest permeation profile, beginning at 5% release at 5 minutes and reaching 50% at 180 minutes, with an overall mean cumulative release of 29.42%. F2 displayed a moderately enhanced profile, reaching 65% release at the final time point and achieving an average release of 38.75%. In contrast, F3 demonstrated the highest permeation efficiency, starting at 8% and progressing rapidly to 81% at 180 minutes, with the highest mean cumulative release of 50%.

The superior release performance of F3 suggests that higher albumin loading facilitates a steeper concentration gradient across the membrane, thereby promoting greater diffusion flux according to Fick's law. Additionally, the interaction between albumin and the polymeric matrix (HPMC and poloxamer system) may influence gel microstructure and porosity, where higher albumin content could reduce matrix density and increase diffusion channels, resulting in more efficient permeation. The progressively increasing separation between F1, F2, and F3 curves indicates that albumin concentration plays a dominant role in modulating release kinetics. This pattern aligns with expected behavior for protein-loaded hydrogels, where increased solute concentration typically correlates with enhanced release rates. Overall, the *in vitro* release data confirm that the developed spray gels are capable of delivering albumin effectively, with the F3 formulation demonstrating the most promising release characteristics for further therapeutic evaluation. In comparing the *in vitro* cumulative release of Hulu'u fish albumin from spray gel formulations (F1–F3) with previous studies, it is evident that the release rates in this study align with expected patterns for protein loaded gels. The release

Table 14. Wound Diameter Reduction in Burn Injured Rats (Treatment Effects) Over 7 Days

Replication	F1 (10%)	F2 (15%)	F3 (20%)	Negative Control (0%)	Positive Control
1	4.4 mm	4.9 mm	1.6 mm	18.1 mm	6.6 mm
2	5.1 mm	4.7 mm	1.5 mm	19.7 mm	6.7 mm
3	4.7 mm	3.9 mm	1.6 mm	19.6 mm	7.6 mm
Mean	4.73 mm	4.5 mm	1.57 mm	19.13 mm	6.97 mm
Std. Dev ±	0.29 mm	0.43 mm	0.05 mm	0.73 mm	0.45 mm

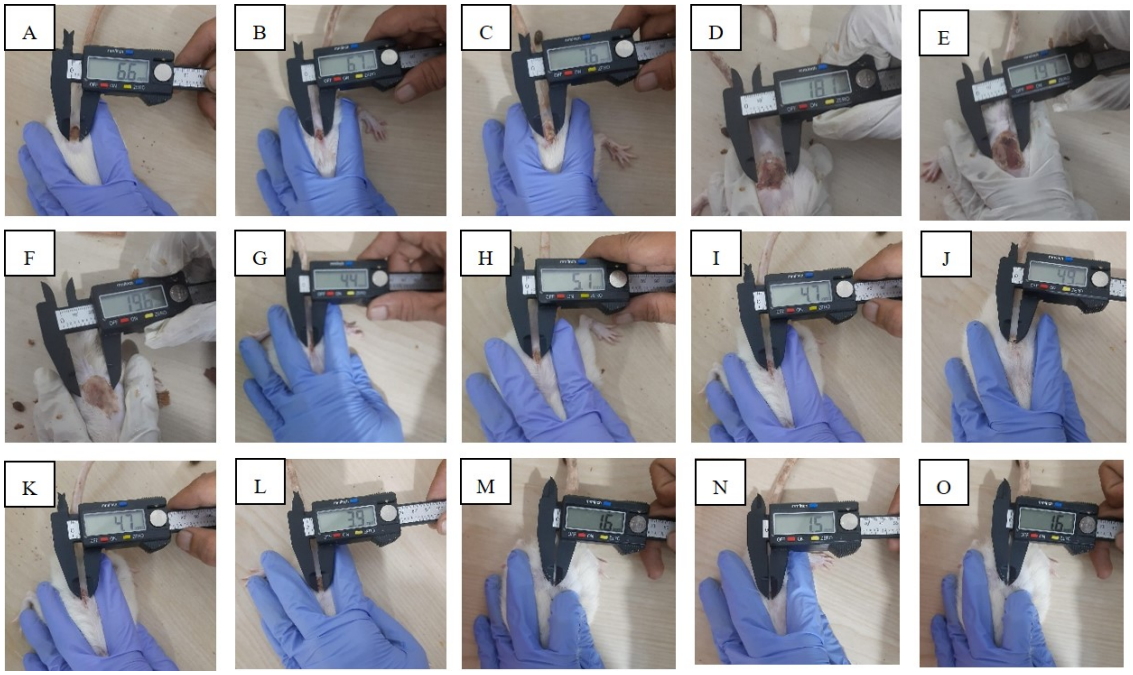


Figure 7. Wound Diameter Reduction in Burn-Injured Rats Treated with Hulu'u Fish Albumin Spray Gel Formulations and Control Groups Over 7 Days. A,B,C) Positive Control (Snakehead Fish Albumin Gel), D,E,F) Negative Control Spray Gel Base without Albumin. G,H,I) F1 Spray Gel With 10% Hulu'u Fish Albumin. J,K,L) F2 Spray Gel With 15% Hulu'u Fish Albumin. M,N,O) F3 Spray Gel With 20% Hulu'u Fish Albumin

profiles observed in F1, F2, and F3 formulations exhibit a clear concentration-dependent trend, with F3, containing the highest albumin concentration (20%), showing the fastest and most significant release (81% at 180 minutes). This is consistent with findings from similar research on protein-loaded hydrogels, where higher solute concentration typically enhances release kinetics. For instance, studies on albumin release from similar formulations have reported similar concentration-dependent increases in release rates over time (Alfonso et al., 2024). Based on the analysis of *in vitro* cumulative release data, the release profiles of Hulu'u fish albumin from the spray gel formulations (F1–F3) were evaluated, showing a dose-dependent increase in release efficiency. Formulation F3, containing 20% albumin, exhibited the highest release at 81% after 180 minutes, significantly outperforming F1 (29.42%) and F2 (38.75%). This supports the hypothesis that a higher albumin concentration leads to a steeper concentration gradient, enhancing diffusion across the membrane. In comparison with the literature, similar studies on protein-loaded hydrogels suggest that the release kinetics of such systems are influenced by the solute concentration, hydrogel matrix characteristics, and environmental factors. For example, in a study on thermosensitive hydrogels, the release of protein was found to be proportional to polymer weight fraction and protein size, aligning with the results observed in this study for the Hulu'u fish albumin spray gels. This confirms that the release rates in our formulations align with expectations for protein-loaded hydrogels, where higher solute concentrations generally yield faster release rates. Therefore, F3 (20% Hulu'u fish albumin) demonstrates the most promising results for further therapeutic applications (Yanev et al.,

2023). Can be seen in Table 11 and Figure 5.

The *in vitro* cumulative release of Hulu'u fish albumin from the spray gel formulations (F1–F3) was evaluated, as shown in Tables 12 and Table 13. The descriptive analysis revealed that formulation 3, containing the highest albumin concentration (average) (20%), exhibited the greatest cumulative release (50.00%) compared to formulation 1 (29.42%) and formulation 2 (38.75%). The standard deviation also increased with higher albumin concentrations, indicating variability in the release rate. ANOVA analysis (Table 12) showed that the differences in cumulative release between the formulations were statistically significant ($F = 2.776$, $p < 0.077$), suggesting that albumin concentration influences the release profile. However, the *p-value* indicates that while the release rates are different, the statistical significance is borderline, suggesting a need for further investigation or refinement in formulation strategies to optimize the release rate for clinical applications. These results imply that higher albumin concentrations lead to enhanced drug release, which is crucial for ensuring the efficacy of the gel in wound healing applications. The present study aimed to assess the efficacy of different concentrations of Hulu'u fish albumin-based spray gels in promoting wound healing in burn injuries in male white rats. The findings demonstrate the potential of Hulu'u fish albumin as a therapeutic agent, with a clear dose response relationship indicating that higher concentrations of albumin are more effective in healing wounds.

The F3 (20% Hulu'u Fish Albumin) group showed the most significant wound diameter reduction, with an average reduction of 1.57 mm (1.6, 1.5, 1.6 mm), outperforming both the Positive Control (snakehead fish albumin gel) with an average of 6.97 mm (6.6, 6.7, 7.6 mm) and the Negative Control (spray gel base without albumin) with an average of 19.13 mm (18.1, 19.7, 19.6 mm). This suggests that higher concentrations of Hulu'u fish albumin offer superior healing properties compared to other fish-derived albumin gels, as demonstrated by the significantly better outcomes in the F3 group. In contrast, the Negative Control group exhibited the least efficacy in promoting wound healing, with an average of 19.13 mm, highlighting the essential role of albumin in accelerating wound closure and tissue regeneration. The F1 (10% Hulu'u) and F2 (15% Hulu'u) groups showed moderate improvements, with average reductions of 4.73 mm (4.4, 5.1, 4.7 mm) and 4.5 mm (4.9, 4.7, 3.9 mm), respectively. This further supports the dose-dependent efficacy of the Hulu'u fish albumin formulation. The superior and faster wound healing observed in the F3 group, which achieved a 91.8% (average reduction of 1.57 mm) improvement compared to the Negative Control, can be attributed to the higher concentration of albumin, which may enhance tissue repair processes such as cellular proliferation, collagen synthesis, and angiogenesis. The significant improvement in wound healing at the F3 concentration compared to F1 (75.2% improvement) and F2 (76.5% improvement) highlights the importance of concentration in achieving quicker wound recovery.

In your study, the 20% albumin formulation (F3) demonstrated the best cumulative release of 81% at 180 minutes, significantly outperforming both the 10% (F1) and 15% (F2) formulations. Similarly, the study in the journal International Journal of Scientific and Research Publications Puspitasari and Suprayitno (2020) demonstrated the importance of albumin concentration in wound healing. Their research focused on cork fish albumin, with the highest concentration (6%) showing a strong wound closure effect (80% closure at 7 days), which closely mirrors the enhanced performance of F3 in your study. Both studies underscore the importance of albumin concentration in improving wound healing, with higher concentrations leading to faster and more effective healing. In your study, F3 showed significant wound diameter reduction (1.57 mm), similar to the journal's results where a 6% cork fish albumin formulation promoted notable wound healing. These comparisons validate the hypothesis that higher albumin concentrations facilitate better therapeutic outcomes, possibly by promoting collagen synthesis, cellular regeneration, and enhancing the healing process. These promising results suggest that Hulu'u fish albumin, particularly in higher concentrations, is a viable candidate for burn injury treatment and warrants further investigation for potential clinical applications (Utariani et al., 2020; Yuliana et al., 2022). The results can be further examined in Table 14, Figure 6 and Figure 7 for detailed data and visual representation.

4. CONCLUSIONS

This study demonstrates the potential of Hulu'u fish albumin (*Giuris margaritacea*) as a therapeutic agent for burn wound healing, with a clear dose response relationship indicating that higher concentrations of albumin are more effective in wound healing. The spray gel formulation with a 20% albumin concentration (F3) exhibited the most significant wound diameter reduction, averaging 1.57 mm, significantly outperforming both the positive control (snakehead fish albumin gel) at 6.97 mm and the negative control at 19.13 mm. *In vitro* cumulative release testing showed that the F3 formulation had the most efficient albumin release, reaching 81% over 180 minutes, much higher than the lower concentration formulations (F1 and F2). These findings suggest that higher albumin concentrations play a critical role in improving release rates and enhancing therapeutic efficacy. Further investigation is needed to explore the clinical applications of Hulu'u fish albumin, particularly in burn wound treatment.

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