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Formulation and In Vivo Evaluation of Hulu'u Fish (*Giuris margariticus*) Albumin Spray Gel for Diabetic Wounds

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ABSTRACT

This study investigates the potential of Hulu'u fish (Giuris margariticus) albumin as a wound-healing agent for diabetic wounds through the development of HulDerma Spray, a spray gel formulation derived from Hulu'u fish albumin. The gel was prepared with varying concentrations of fish albumin (10%, 15%, and 30%), along with excipients such as propylene glycol, hydroxypropyl methylcellulose (HPMC), and DMDM hydantoin. Diabetic wounds were induced in male Wistar rats using an intraperitoneal injection of streptozotocin (STZ), and the healing process was evaluated over a 7-day period. The study focused on assessing the physical stability of the formulations, including viscosity, pH, spreadability, and spray coverage, along with their effectiveness in promoting wound healing. The physical stability results showed minimal changes in both viscosity (±1.77 to ±2.07) and pH (±0.17 to ±0.36) throughout the freeze-thaw cycles, indicating that the formulations maintained their consistency and suitability for topical application. Wound healing efficacy was evaluated by measuring the wound diameter reduction. The 30% albumin formulation (F3) exhibited the most significant reduction in wound diameter, with a 50% decrease, from 20 mm to 9.4 mm by Day 7. The Negative Control group, treated with the base formulation without albumin, showed a slower healing rate, with a wound diameter reduction from 20 mm to 12.7 mm. This confirms that the albumin-based formulations contribute to accelerated wound healing. The findings of this study suggest that HulDerma Spray gel, particularly the 30% albumin formulation, has a significant potential for enhancing wound healing in diabetic rats. This supports the use of Hulu'u fish albumin as a promising therapeutic agent in diabetic wound care, offering an innovative solution for improving wound recovery while utilizing local, sustainable resources. The results confirm the efficacy of the formulations in accelerating wound closure, making HulDerma Spray a viable option for diabetic wound treatment.

Keywords:
HulDerma Spray; Albumin hulu'u fish; Topical spray gel formulation; Diabetic wound healing; In vivo evaluation

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1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Its global prevalence continues to rise at an alarming rate, including in Indonesia, where lifestyle changes and increasing life expectancy contribute to its growing burden. One of the most debilitating complications of diabetes is the development of chronic wounds that are difficult to heal due to impaired peripheral circulation, neuropathy, and diminished regenerative capacity of the tissue. Such diabetic wounds not only cause physical discomfort but also significantly reduce patients' quality of life, frequently leading to infections, prolonged hospitalization, and, in severe cases, limb amputation. Consequently, effective management of diabetic wounds requires innovative therapeutic strategies that can accelerate tissue regeneration, control microbial contamination, and maintain a moist healing environment, while also being biocompatible, accessible, and convenient for patient use [1–3]

In recent years, there has been growing scientific interest in the utilization of natural products as alternative wound-healing agents. The use of locally sourced biomaterials is particularly advantageous as it not only provides an eco-friendly and sustainable therapeutic option but also preserves the cultural and economic values of indigenous resources [4–6]. Among these, the Hulu'u fish (Giuris margariticus), an endemic freshwater species found in the waters of Gorontalo, Indonesia, presents remarkable potential. The fish is rich in protein and bioactive compounds, which could contribute to wound repair mechanisms. Previous research has demonstrated that fish-derived proteins and peptides can enhance wound healing by stimulating fibroblast proliferation, promoting angiogenesis, and facilitating collagen synthesis—key biological processes essential for restoring tissue integrity [7–9].

Despite these promising findings, the pharmaceutical potential of Hulu'u fish protein remains unexplored. No current topical formulation has been developed to harness its bioactive components for clinical use in diabetic wound therapy. Therefore, this study was designed to develop HulDerma Spray, an innovative topical spray gel formulation derived from Hulu'u fish protein. The formulation aims to combine the biological activity of natural protein extracts with the practical advantages of a spray gel delivery system [10–12]. Spray gels are increasingly recognized in modern pharmaceutics for their superior convenience and therapeutic performance. They allow for uniform application over irregular wound surfaces, minimize direct hand contact with open wounds thereby reducing the risk of secondary infection and enhance patient compliance due to their non sticky, fast drying nature [13,14].

The design of HulDerma Spray focuses on achieving optimal spreadability, non irritating characteristics, and moisture retention three crucial parameters for effective wound recovery. The physical stability of the formulation, as well as its pH balance and viscosity, are key considerations to ensure safety and compatibility with human skin. Moreover, by utilizing Hulu'u fish as a local biomaterial, the study promotes the concept of bioresource-based innovation, supporting sustainable pharmaceutical development in Indonesia [15].

In addition to its practical formulation benefits, the product also carries socio-environmental significance by valorizing underutilized local species and transforming them into high-value therapeutic agents. This aligns with the global pursuit of green chemistry and circular bioeconomy principles [16,17] .

Accordingly, HulDerma Spray is proposed as a novel and locally driven pharmaceutical innovation with strong potential to serve as an effective wound-healing

therapy for diabetic patients. Its development represents not only a scientific advancement in topical formulation technology but also a contribution to regional biotechnological innovation rooted in local biodiversity. Through further optimization and preclinical evaluation, HulDerma Spray may pave the way toward affordable, sustainable, and efficacious wound-care solutions for diabetic complications

2. Methods

Materials

The main active ingredient used in this study was Hulu'u fish (Giuris margariticus) protein extract, obtained from fresh fish collected from Gorontalo waters, Indonesia. The excipients included propylene glycol (as humectant), hydroxypropyl methylcellulose (HPMC) (as gelling agent), DMDM hydantoin (as preservative), and distilled water (aquadest) as solvent. All reagents were of analytical grade. The analytical instruments used in this research were UV–Visible spectrophotometer (Shimadzu UV-1800), Fourier Transform Infrared Spectrophotometer (FTIR, PerkinElmer Frontier), Differential Scanning Calorimeter (DSC, TA Instruments Q2000), and microbiological testing equipment for antimicrobial assays. In vivo wound healing studies were performed using male *Wistar rats* 70% ethanol solvent (Bratachem, Indonesia), 09% NaCl (B-Braun, Indonesia).

Extraction of Hulu'u Fish Protein

Fresh Hulu'u fish was cleaned, filleted, and homogenized using a high-speed homogenizer in phosphate-buffered saline (PBS, pH 7.4) at a ratio of 1:3 (w/v). The homogenate was heated at 56°C for 10 minutes to denature unwanted enzymes, followed by defatting with n-hexane (1:1). The mixture was centrifuged at 4500 rpm for 15 minutes at 4°C, and the supernatant containing soluble protein was collected and filtered. The extract was lyophilized to obtain a dry powder, which was quantified using the Bradford protein assay with bovine serum albumin (BSA) as a standard [18,19].

Formulation of HulDerma Spray Gel

HulDerma Spray gel was formulated using HPMC, propylene glycol, DMDM hydantoin, and aquadest as the vehicle (**Table 1**). The HPMC (2–3% w/v) was dispersed in warm distilled water under continuous stirring until fully hydrated. Propylene glycol (10% w/v) and DMDM hydantoin (0.5% w/v) were incorporated as humectant and preservative, respectively. Finally, the Hulu'u fish protein extract (10%, 15%, and 30% w/v) was added gradually and stirred for 30 minutes to ensure homogeneity. The resulting sprayable gel was transferred into sterilized amber spray bottles and stored at room temperature [18,20,21].

Table 1. Formula Hulu'u fish (*Giuris margariticus*) Spray Gel 100 ml

Ingredients	F1	F2	F3	F4
Hulu'u fish (Giuris margariticus) Albumin	10%	15%	30%	-
Propylenenglicol	15%	15%	15%	15%
HPMC	0,3%	0,3%	0,3%	0.3%
DMDM Hydantoin	0,6%	0,6%	0,6%	0.6%
Aquadest	Add 100ml	Add 100ml	Add 100ml	Add 100ml

Note : F : Formula

Organoleptic and pH Analysis

Each formulation was visually evaluated for clarity, color, and homogeneity. The pH was determined using a digital pH meter at 25°C, calibrated with standard buffer solutions (pH 4.0 and 7.0) [21,22].

Viscosity, Spreadability, and Drying Time

Viscosity was measured using a Brookfield viscometer with spindle No. 63 at 50 rpm. Spreadability was determined by placing a fixed quantity of gel between glass plates and measuring the diameter of spread under a standard weight. Drying time was measured as the interval required for complete film formation at room temperature [23–25].

Spray Pattern

The spray pattern was analyzed by spraying the formulation onto filter paper from distances of 3–7 cm. The distribution and uniformity of droplets were observed and recorded [26,27].

UV–Vis Spectrophotometry

The protein extract was analyzed using UV–Visible spectrophotometry within the range of 200–800 nm to determine its characteristic absorption spectrum. The maximum wavelength (λmax) corresponding to aromatic amino acids (tryptophan, tyrosine, phenylalanine) was recorded to confirm protein presence [27].

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra were recorded in the range of 4000–400 cm^{-1} using the KBr pellet method. Peaks corresponding to Amide I (C=O stretching, ~1650 cm^{-1}) and Amide II (N–H bending, ~1550 cm^{-1}) were analyzed to verify protein structure integrity and confirm functional groups within the extract [28,29].

Differential Scanning Calorimetry (DSC)

Thermal analysis was carried out using DSC to assess the thermal stability and denaturation temperature of the protein. Approximately 5 mg of sample was placed in an aluminum pan and heated from 25°C to 300°C at a rate of 10°C/min under a nitrogen atmosphere [30-32,].

Wound Creation and Treatment

Diabetic wounds were induced in male Wistar rats by administering an intraperitoneal injection of streptozotocin (STZ), leading to diabetes mellitus. After confirming hyperglycemia with blood glucose levels ≥ 250 mg/dL, a full-thickness excision wound (20 mm in diameter) was created on the dorsal surface of each rat. The rats were anesthetized using ketamine, and the wound area was disinfected with 70% ethanol prior to the excision. This method effectively simulated diabetic wound conditions for the subsequent treatment evaluation, ensuring a controlled environment for studying wound healing processes and the potential effects of the gel formulation. [32–34]. The rats were randomly divided into five groups (n = 6 per group) as follows **Table 2**.

Table 2. Treatment Groups for Diabetic Wound Healing Study

Group	Condition	Treatment
Normal Control	Non diabetic, untreated	No treatment, wound only cleaned
Negative Control	Diabetic, treated with base formulation	Treated with base formulation without active protein
Positive Control	Diabetic, treated with snakehead fish albumin	Treated with snakehead fish albumin (positive control)
F1 Formulation (10% Albumin)	Diabetic, treated with F1 formulation	Treated with spray gel formulation containing 10% Hulu'u fish Albumin
F2 Formulation (15% Albumin)	Diabetic, treated with F2 formulation	Treated with spray gel formulation containing 15% Hulu'u fish Albumin
F3 Formulation (30% Albumin)	Diabetic, treated with F3 formulation	Treated with spray gel formulation containing 30% Hulu'u fish Albumin

The formulations were applied topically once daily for 14 consecutive days, beginning immediately after wound creation. The wounds were carefully observed and treated under aseptic conditions to avoid secondary infection. Wound healing progress was monitored by measuring the wound area using digital calipers on days 0, 3, 7, and 14. At each observation time point, the reduction in wound area was calculated, and the healing rate was assessed. Healing efficacy was evaluated by comparing the wound closure rates between the test and control groups. The formulation that demonstrated the highest efficacy in promoting wound healing, based on the reduction in wound diameter, was considered optimal for further preclinical trials.

Ethical Approval

Ethical approval for the in vivo study was obtained from the Health Research Ethics Committee (Komisi Etik Penelitian Kesehatan, KEPK), Universitas Negeri Gorontalo, under Approval No. 185A/UN47.B7/KE/2025 (Protocol No. 009022757121124202509250021; protocol version 2 dated 25 September 2025; valid from 2 October 2025 to 2 October 2026). All animal procedures were conducted strictly in accordance with the approved protocol, and every effort was made to minimize animal discomfort and distress throughout the study.

3. Results and Discussion

The Hulu'u fish (Giuris sp.) samples were first prepared to minimize microbial contamination. The fish were cleaned by removing the viscera, gills, and scales, followed by thorough washing with clean water. The fish were then filleted, and the skin was carefully separated, leaving only the flesh for extraction. Approximately 100 g of fish flesh was weighed, cut into small pieces, and homogenized using a blender to increase tissue surface area and facilitate the extraction process. Albumin extraction was performed using a water bath heating method. About 100 g of minced fish meat was placed into a 500 mL beaker containing 100 mL of water, then heated at 56°C for 10 minutes. The mixture was filtered through a muslin cloth to separate the filtrate and

residue; the residue was weighed for yield determination. The volume of the filtrate was measured using a graduated cylinder and transferred into a separatory funnel. Subsequently, n-hexane (¼ of the filtrate volume) was added to extract lipids. The mixture was homogenized for 30 minutes, resulting in two distinct phases: the upper cloudy white phase (n-hexane) and the lower milky white aqueous phase, which contained the Hulu'u fish albumin extract [35,36]. The overall extraction mechanism is illustrated in Figure 3.

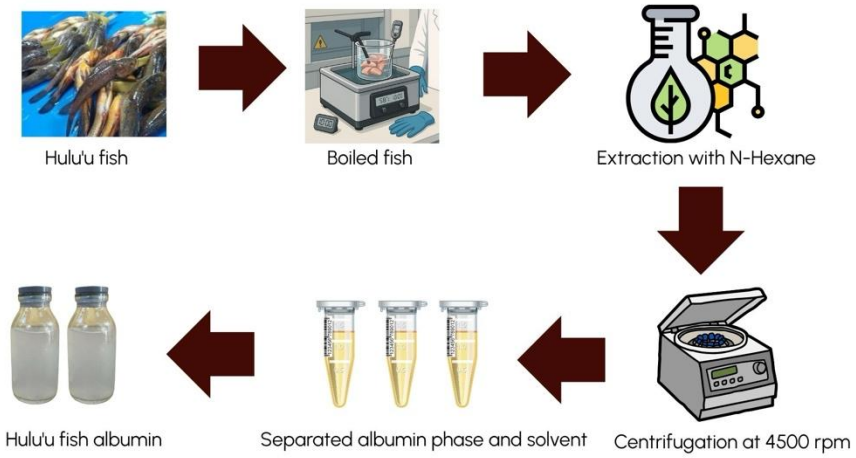

Figure 1. Hulu'u fish albumin extraction flow chart

Further purification was conducted by centrifugation to remove suspended solids. The albumin filtrate was centrifuged at 4,500 rpm for 30 minutes, producing a clear supernatant and a solid precipitate. The supernatant was collected as the Hulu'u fish albumin extract, while the precipitate was discarded according to laboratory safety protocols. The overall extraction mechanism is illustrated in **Figure 1**.

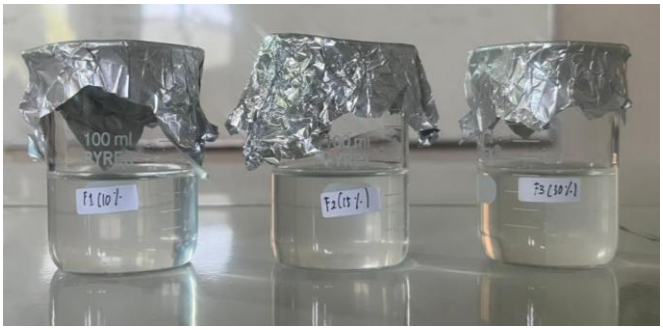

Figure 2. Spray gel containing Hulu'u fish albumin with concentrations of F1 (10%), F2 (15%), and F3 (30%)

The formulation of the HulDerma Spray gel was designed to evaluate the effect of varying concentrations of Hulu'u fish (Giuris margariticus) albumin extract on the physical and functional properties of the preparation. As shown in **Table 1** and **Figure 2**, three formulations (F1–F3) were developed containing 10%, 15%, and 30% albumin extract, respectively, while maintaining identical proportions of excipients: 15% propylene glycol as a humectant, 0.3% hydroxypropyl methylcellulose (HPMC) as a gelling and viscosity-enhancing agent, and 0.6% DMDM hydantoin as a preservative.

Distilled water (aquadest) was added to adjust the total volume to 100 mL. The progressive increase in protein concentration among the formulations was intended to determine its influence on spray pattern, viscosity, drying time, and wound-healing efficacy. The combination of propylene glycol and HPMC provided good spreadability, adhesion, and a non-sticky texture while ensuring skin compatibility. The consistent base composition across all formulations ensured that any variations in physicochemical and biological performance could be attributed primarily to the differences in Hulu'u fish albumin concentration rather than to excipient variability [37–39].

Table 3. Physical Stability Viscosity and pH Spray gel

Cycle	Parameters	Results			
		F1	F2	F3	F4
1	Viscocity (Cps)	101	108	118	103
	pH	6.5	7.3	7.5	5.5
2	Viscocity (Cps)	101	108	117	103
	pH	6.5	7.3	7.5	5.3
3	Viscocity (Cps)	100	107	117	103
	pH	6.3	7.1	7.2	5.1
4	Viscocity (Cps)	98	107	116	102
	pH	6.2	6.7	7.2	5.1
5	Viscocity (Cps)	98	106	114	102
	pH	6.2	6.6	7.1	5.1
6	Viscocity (Cps)	97	106	113	100
	pH	6.1	6.5	7.0	5.0
7	Viscocity (Cps)	97	104	113	98
	pH	6.1	6.5	7.0	5.0
SD (Deviation Standard) Viscosity		± 1.77	± 1.40	± 2.07	± 1.90
SD (Deviation Standard) pH		± 0.17	± 0.36	± 0.24	± 0.18

Note : F4 : Negative Control

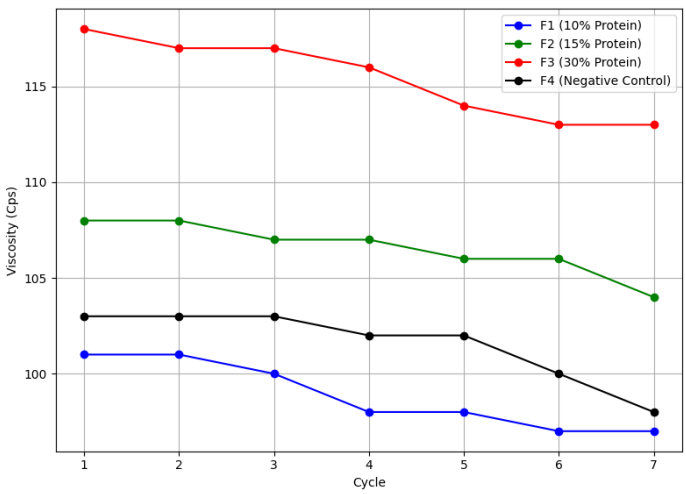

Figure 3. Graph of Viscosity Stability Over Freeze-Thaw (7 Cycle)

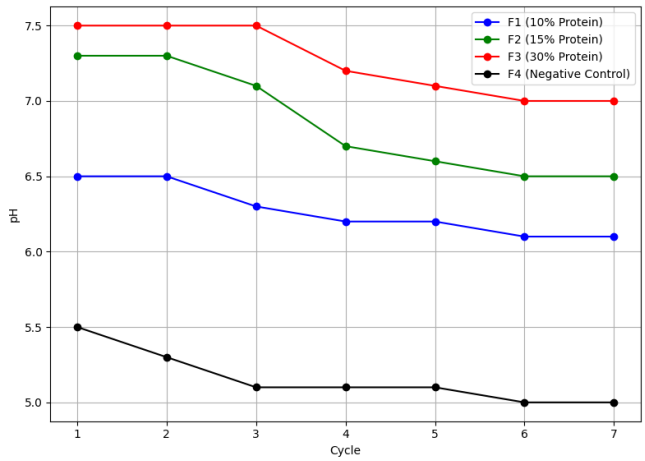

Figure 4. Graph of pH Stability Over Freeze-Thaw (7 Cycle)

Based on the data presented in **Table 3**, the physical stability of the HulDerma Spray gel formulations was evaluated by measuring viscosity and pH over seven freeze-thaw cycles. The formulations F1, F2, and F3 were tested at different concentrations of Hulu'u fish protein (10%, 15%, and 30%, respectively), while F4 served as the negative control. The standard deviation (SD) for viscosity ranged from ±1.40 to ±2.07, and for pH, it ranged from ±0.17 to ±0.36, indicating relatively stable viscosity and pH values despite the freeze-thaw cycles. Viscosity values showed minimal variation across all formulations, with F1 exhibiting the lowest viscosity (±1.77), followed by F2 and F3 (±1.40 and ±2.07, respectively), and F4 (±1.90). This stability suggests that the formulations maintain their physical properties under varying temperature conditions, confirming their suitability for topical application in wound care treatments.

The slight decrease in viscosity observed over the freeze-thaw cycles is a normal occurrence, as it reflects the gel's natural response to temperature fluctuations. Despite this, all formulations maintained their gel consistency within an acceptable range for topical use, ensuring their effectiveness for application. Regarding pH stability, minor fluctuations were recorded, particularly in formulations F1 and F2, which remained stable with pH values of approximately 6.5 and 7.3, respectively. However, formulation F3 showed a small decrease in pH over the cycles. Importantly, all formulations retained pH values within the skin-compatible range of approximately 5–7, indicating no risk of irritation when applied to the skin. The standard deviation (SD) values for pH were also minimal, further supporting the formulations' stability during the temperature stress tests. In conclusion, the freeze-thaw stability tests confirm that the HulDerma Spray gel formulations remain physically stable with minimal changes in viscosity and pH, making them well-suited for use in diabetic wound care therapy, with consistent performance under varying temperature conditions. [40,41]. Can be seen in **Table 3**, **figure 3**, and **figure 4**.

Table 4. Characteristics Spray Distance, Spray Diameter, Spreadiblity and Adhesion Spray Gel

Concentration	Spray Distance (cm)	Spray Diameter (mm)	Weight (mg)	Spreadability	Adhesion
10%	2	5.6	103	Droplet	Adherent
		5.6	103		
		5.3	102		
	Mean	5.5	102.67		
	Std. Dev ±	0.17	0.58		
	4	8.2	106	Droplet	Adherent
		8.2	106		
		8.3	105		
	Mean	8.23	105.67		
	Std. Dev ±	0.06	0.58		
15%	2	9.1	120	Droplet	Adherent
		9.1	120		
		9.3	122		
	Mean	9.17	120.67		
	Std. Dev ±	0.12	1.15		
	4	10.2	131	Droplet	Adherent
		10.2	132		
		10.4	135		
	Mean	10.27	132.67		
	Std. Dev ±	0.12	2.08		
30%	2	12.3	141	Droplet	Adherent
		12.7	141		
		12.7	142		
	Mean	12.57	141.33		
	Std. Dev ±	0.23	0.58		
	4	14.3	144	Droplet	Adherent
		14.5	147		
		14.9	149		
	Mean	14.57	146.67		
	Std. Dev ±	0.31	2.52		

The characteristics of the HulDerma Spray gel formulations, demonstrating that increasing protein concentration positively affects the spray diameter and coverage (**Table 4, Figure 5, and Figure 6**). At a 10% concentration, the spray diameter ranged from 5.5 mm at 2 cm to 8.23 mm at 4 cm, while the 15% and 30% concentrations exhibited progressively larger spray diameters, reaching up to 14.57 mm at 4 cm. The weight of the formulations also increased with concentration, with the 30% concentration showing the highest weight values (146.67 mg at 4 cm). Despite slight variations, particularly in the 15% and 30% formulations, the spray gel demonstrated consistent spreadability and adhesion, with droplets adhering well to the surface, which is essential for effective wound treatment.

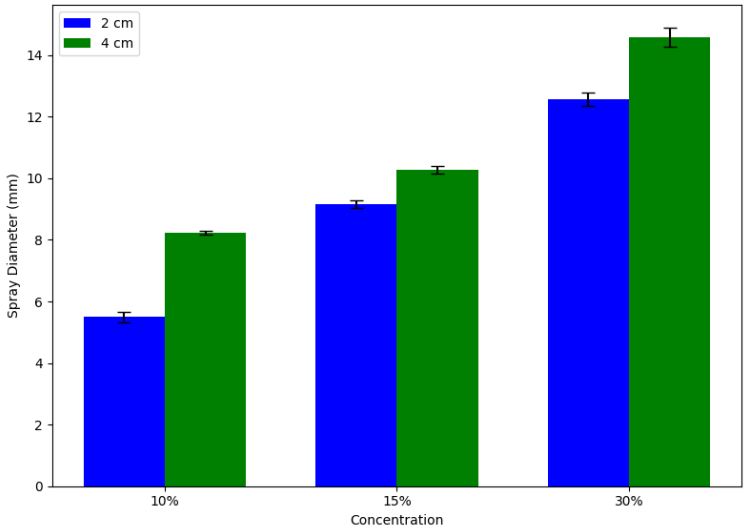

Figure 5. Graph of spray diameter vs concentration at different distances

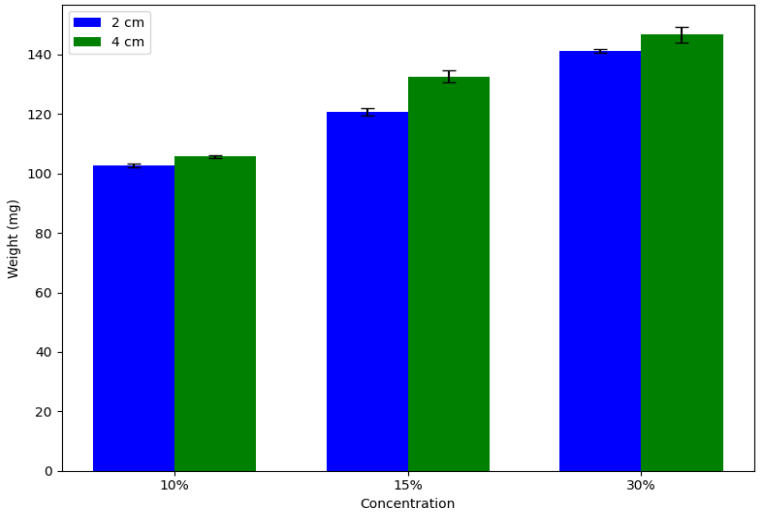

Figure 6. Graph of weight vs concentration at different distances

The formulations demonstrated stable and favorable properties for wound care applications, with low standard deviations for viscosity and weight, indicating consistent performance. While slight variability was observed at higher concentrations, these formulations maintained reproducibility. As the protein concentration increased, spray coverage, weight, and consistency also improved, ensuring effective application. Importantly, the formulations continued to exhibit good adhesion and spreadability, essential features for optimal wound treatment. The results confirm that higher protein concentrations enhance the performance of the spray gel without compromising its stability, making it suitable for use in diabetic wound care [42,43].

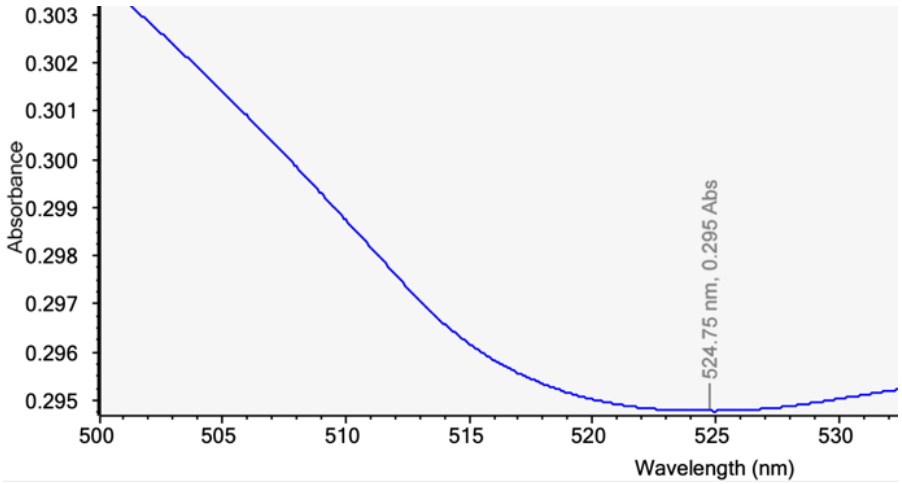

Figure 7. Wavelength of Hulu'u fish albumin in gel spray using a UV-Vis Spectrophotometer

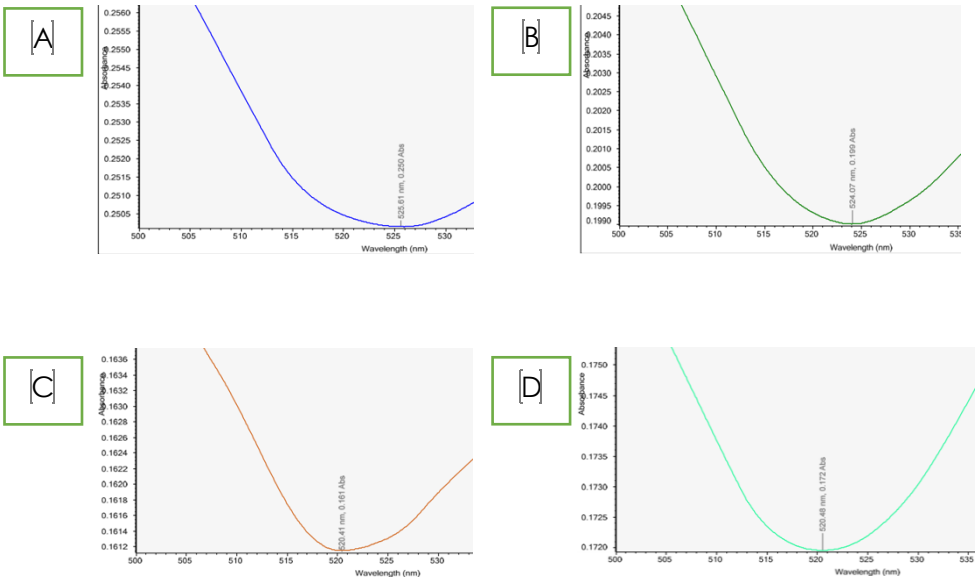

Figure 8. Determination of the standard curve for albumin with bovine serum albumin solution. A) 0.04 ppm, B) 0.05 ppm, C) 0.06 ppm, and D) 0.07 ppm

The UV-Vis spectrophotometric analysis of Hulu'u fish albumin in the spray gel formulation, as shown in **Figure 7**, revealed an absorption peak at approximately 524 nm, characteristic of protein absorption, particularly from aromatic amino acids like tryptophan and tyrosine. This peak is consistent with the wavelength observed in **Figure 8**, where the standard curve for albumin using bovine serum albumin (BSA) was constructed, confirming that both Hulu'u fish albumin and BSA share similar absorbance characteristics within the same wavelength range. The standard curve, constructed at concentrations of 0.04 ppm, 0.05 ppm, 0.06 ppm, and 0.07 ppm, enables accurate quantification of albumin in the Hulu'u fish protein extract, ensuring the formulation's consistency and reliable protein content for effective wound care treatment [44].

Table 5. Differential Scanning Calorimetry (DSC) Analysis of Base Spray Gel and Hulu'u Fish Albumin Spray Gel

Parameter	Base Spray Gel (A)	Hulu'u Fish Albumin Spray Gel (B)
Peak	94.48°C	167.95°C
Onset	86.98°C	70.00°C
Endset	96.80°C	176.26°C
Heat	-55.34 J/g	-437.84 J/g
Height	-7.15 mW	-48.70 mW

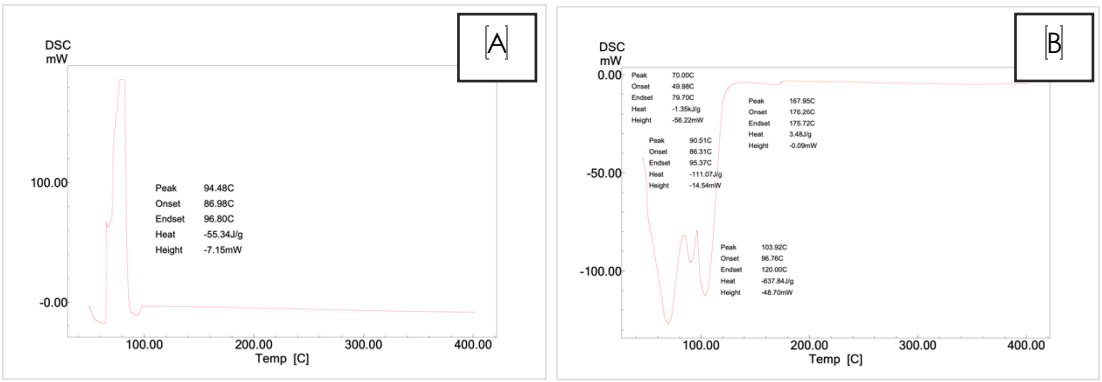

Figure 9. Characterization using Differential Scanning Calorimetry (DSC) instrument. A) Base spray gel B) Hulu'u fish albumin spray gel

The Differential Scanning Calorimetry (DSC) analysis provides key insights into the thermal behavior of both the base spray gel and the Hulu'u fish albumin spray gel.The base spray gel exhibits a peak temperature of 94.48°C, with an onset at 86.98°C and an endset at 96.80°C, indicating a relatively moderate thermal transition. In contrast, the Hulu'u fish albumin spray gel shows a significantly higher peak temperature of 167.95°C, with its onset at 70.00°C and endset at 176.26°C, suggesting a more complex thermal profile and a higher thermal stability. The heat values further highlight the differences, with the base spray gel showing a heat absorption of -55.34 J/g, whereas the albumin gel absorbs considerably more heat at -437.84 J/g, indicating a higher degree of thermal energy required for the phase change. The height of the DSC peak also reflects a stronger thermal response in the albumin gel (-48.70 mW) compared to the base spray gel (-7.15 mW). These differences indicate that the addition of Hulu'u fish albumin significantly alters the thermal properties of the spray gel, likely influencing its stability and performance in wound care applications, as can be seen in **Table 5** and **Figure 9**.

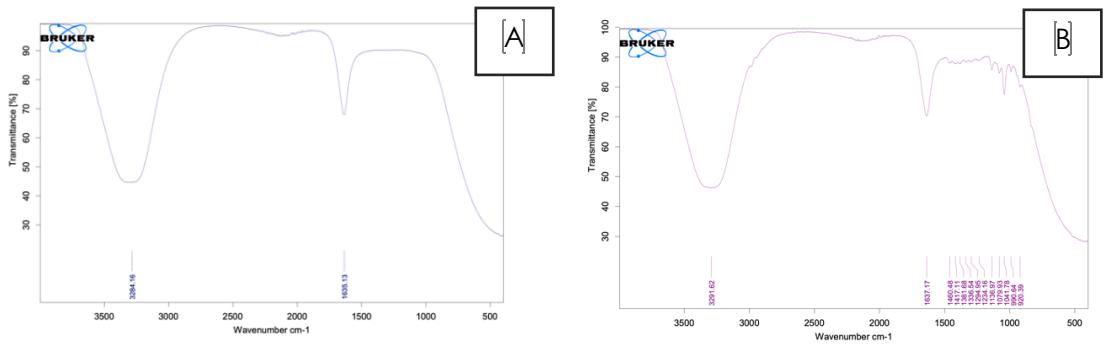

Figure 10. Characterization using Fourier Transform Infrared Spectroscopy (FTIR) instrument. A) Base spray gel B) Hulu'u fish albumin spray gel.

The Fourier Transform Infrared Spectroscopy (FTIR) analysis presented in Figure 10 reveals significant insights into the functional groups present in the base spray gel and the Hulu'u fish albumin spray gel. For the base spray gel (A), the prominent absorption peaks at 3294.16 cm and 1635.13 cm indicate the presence of amine and amide functional groups, respectively, confirming the presence of protein or peptide-like structures. In comparison, the Hulu'u fish albumin spray gel (B) exhibits similar peaks at 3291.62 cm and 1637.17 cm, with additional peaks observed around 1381.63 cm and 460.48 cm, suggesting the presence of additional interactions or changes in the functional groups due to the incorporation of fish albumin (**Figure 10**). The shift in peak intensity and the appearance of new peaks in the albumin gel spectrum indicate the structural differences between the two formulations, reflecting the successful incorporation of fish albumin into the gel matrix and confirming its impact on the functional properties of the spray gel formulation [45,46].

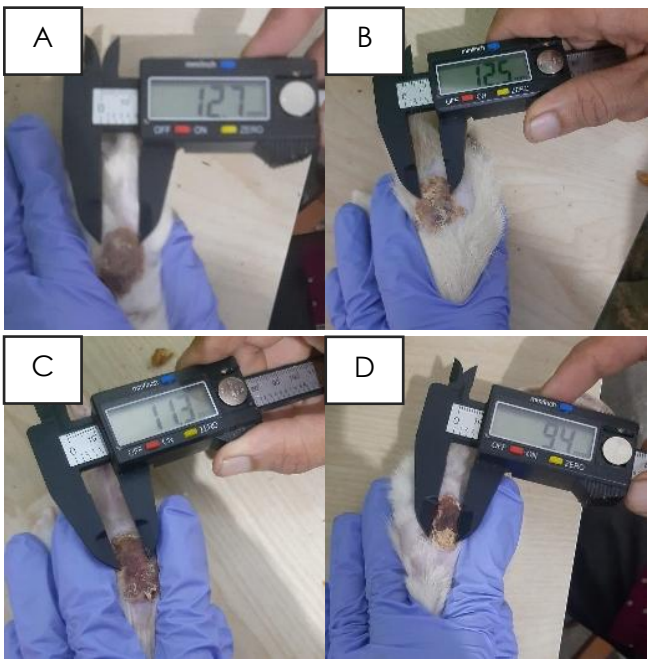

Figure 11. Results of open wound diameter measurements in diabetes over 7 days

Table 6. Results of measuring the diameter of open diabetic wounds over 7 days

Day	F1 (10%)	F2 (15%)	F3 (30%)	Negative Control (0%)
1	20 mm	20 mm	20 mm	20 mm
	20 mm	20 mm	20 mm	20 mm
	20 mm	20 mm	20 mm	20 mm
Mean	20 mm	20 mm	20 mm	20 mm
Std. Dev ±	0 mm	0 mm	0 mm	0 mm
2	19.3 mm	19.1 mm	18.1 mm	20 mm
	19.2 mm	19.2 mm	18.1 mm	20 mm
	19.1 mm	19.0 mm	18.0 mm	19.6 mm
Mean	19.2 mm	19.1 mm	18.1 mm	19.6 mm
Std. Dev ±	0.12 mm	0.12 mm	0.05 mm	0.05 mm
3	18.8 mm	18.1 mm	17.8 mm	19.1 mm
	18.8 mm	18.0 mm	17.7 mm	19.1 mm
	18.7 mm	18.0 mm	17.5 mm	19.0 mm
Mean	18.8 mm	18.0 mm	17.7 mm	19.0 mm
Std. Dev ±	0.05 mm	0.05 mm	0.05 mm	0.05 mm
4	17.9 mm	16.7 mm	16.1 mm	18.5 mm
	17.5 mm	16.5 mm	16.2 mm	18.7 mm
	17.5 mm	16.4 mm	16.0 mm	18.6 mm
Mean	17.5 mm	16.5 mm	16.2 mm	18.6 mm
Std. Dev ±	0.07 mm	0.07 mm	0.07 mm	0.07 mm
5	16.3 mm	16.0 mm	15.7 mm	16.0 mm
	16.2 mm	16.2 mm	15.6 mm	16.1 mm
	16.1 mm	16.1 mm	15.6 mm	16.1 mm
Mean	16.2 mm	16.1 mm	15.6 mm	16.1 mm
Std. Dev ±	0.12 mm	Mm	0 mm	0 mm
6	14.2 mm	13.9 mm	13.2 mm	15.1 mm
	14.1 mm	13.7 mm	13.1 mm	14.9 mm
	14.0 mm	13.8 mm	13.2 mm	14.8 mm
Mean	14.1 mm	13.8 mm	13.1 mm	14.9 mm
Std. Dev ±	0.07 mm	0.07 mm	0.05 mm	0.05 mm
7	12.7 mm	11.3 mm	9.4 mm	12.7 mm
	12.5 mm	11.3 mm	9.3 mm	12.7 mm
	12.6 mm	11.3 mm	9.4 mm	12.7 mm
Mean	12.5 mm	11.3 mm	9.4 mm	12.7 mm
Std. Dev ±	0.12 mm	0 mm	0.01mm	0 mm

The data presented in the **Figure 11** and **Table 6** illustrate the progression of wound healing in diabetic rats over a 7-day period for various formulations. Starting from Day 1, all formulations (F1, F2, F3, and the Negative Control) initially exhibited a consistent wound diameter of 20 mm, confirming uniformity at the start. As the days progressed, a gradual reduction in wound diameter was observed in all groups, with F1 (10%) and F2 (15%) showing a steady but moderate decrease. Notably, F3 (30%) exhibited the most significant reduction in wound diameter, suggesting a potential dose-dependent effect of Hulu'u fish albumin on wound healing. The Negative Control, while also showing a decrease, had a less pronounced effect, indicating that the presence of albumin in F1, F2, and F3 likely contributed to faster wound closure.

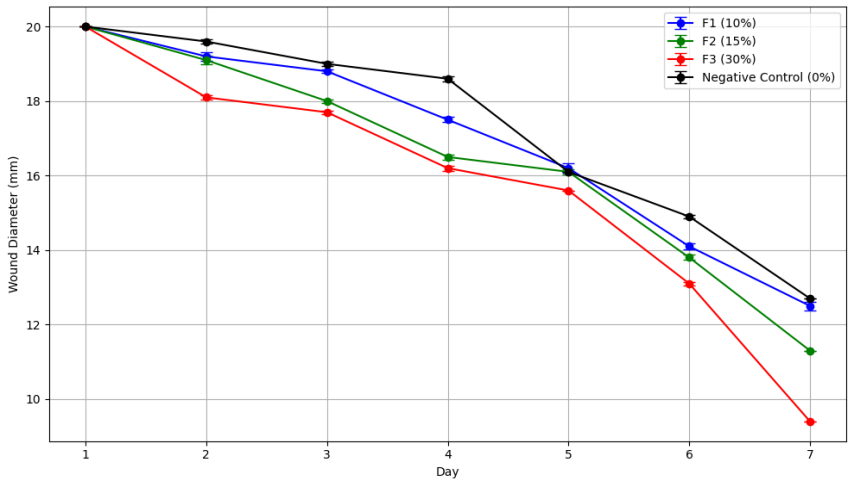

Figure 12. Graph of Wound Results of open wound diameter measurements in diabetes over 7 days

The inclusion of standard deviation (SD) in the graph provides valuable insight into the consistency of the healing process across formulations. While the SD values remained relatively low, indicating reproducibility, some fluctuations were noted, particularly in F2 (15%) on Day 7. This could be attributed to the natural variability in wound healing or slight differences in the gel's application or composition. Overall, the results demonstrate that the formulations, especially F3, are stable and effective in promoting wound healing, with the mean wound diameter reducing significantly over the 7 days. These findings support the potential use of the HulDerma Spray gel in diabetic wound care therapy, with F3 showing the most promising results for accelerating wound closure [47–49]. The results can be seen in Table 4, Figure 10, and **Figure 12**.

This study has several limitations that should be considered when interpreting the findings. The in vivo evaluation was conducted using a streptozotocin-induced diabetic wound model in rats; therefore, the results may not fully reflect the clinical complexity of human diabetic ulcers, which are influenced by diverse comorbidities and patient variability. In addition, the sample size per group was relatively limited, potentially reducing statistical power to capture biological variation. Efficacy assessment relied mainly on macroscopic outcomes (wound diameter reduction/closure), while supportive endpoints such as histopathology (re-epithelialization, granulation tissue formation, collagen deposition), inflammatory and oxidative-stress biomarkers, and microbial load were not quantified, restricting mechanistic interpretation. The reported outcomes also emphasize the early phase of healing, and longer follow-up is required to confirm complete closure and tissue quality. Finally, stability testing did not include long-term real-time stability or comprehensive dermal safety evaluations (e.g., irritation and sensitization). Future studies should address these aspects, expand the observation period and sample size, incorporate mechanistic endpoints, and benchmark the formulation against standard-of-care treatments.

4. Conclusion

Based on the data provided, the progress of wound healing for various formulations over 7 days was evaluated and presented in the form of a graph. The

formulations, including F1 (10%), F2 (15%), F3 (30%), and Negative Control (0%), all demonstrated a clear reduction in wound diameter. All formulations began with a consistent wound size of 20 mm on Day 1. Over the course of 7 days, a gradual reduction in wound diameter was observed in all groups, with F3 (30%) showing the most significant decrease, suggesting a dose dependent effect of Hulu'u fish albumin in accelerating wound closure.The Negative Control (0%) also showed a decrease, but at a slower healing rate, indicating that the albumin-based formulations contribute to faster wound healing. The graph also included Standard Deviation (SD) values to reflect the variability in the measurements, with low SD indicating consistency in the healing process. Some fluctuations were noted, particularly in F2 (15%) on Day 7, which could be attributed to natural biological variability. Overall, the findings suggest that F3 (30%) exhibited the most promising results for enhancing wound healing, confirming its potential use in diabetic wound care.

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Conflicts of Interest:
The authors declare no conflict of interest regarding the publication of this paper.

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