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Research Article

Efficacy and Safety Assessment of Emulgel Containing SnakeheadFish (*Ophiocephalus striatus*) Dry Extract for Burn Wound Treatment in Rabbit Model

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ABSTRACT

Burn injuries are a significant health concern requiring effective treatment to accelerate healing and reduce complications. Conventional topical therapies often have limitations, prompting the development of alternative treatments. Snakehead fish extract, rich in bioactive compounds, has been traditionally used for wound healing. This study aimed to evaluate the efficacy and safety of an emulgel containing snakehead fish dry extract at different concentrations for burn wound treatment in rabbits. Three emulgels were formulated with extract concentrations of 0.25%, 0.5%, and 1%, then characterized for physicochemical properties, including pH, viscosity, spreadability, and adhesion. Protein content was determined using the Bradford method. An in vivo study was performed on rabbits with second-degree burn wounds, divided into five groups: negative control (emulgel base), positive control (Burnazine cream), and treatment groups receiving 0.25%, 0.5%, and 1% extract emulgel. Wound healing was assessed by measuring wound contraction for 16 days. Organoleptic tests showed no changes in color, odor, or homogeneity during four weeks of storage. The emulgel formulations showed stable physicochemical properties, with pH 6.0–6.5, viscosity 8000–10000 cPs, spreadability 6.8–6.9 cm, and adhesion time over 4 seconds, suitable for skin application. Protein content increased proportionally with extract concentration. The in vivo study showed that 1% extract emulgel significantly accelerated wound healing, with greater wound contraction compared to both controls ($p < 0.05$). No irritation, erythema, or edema were observed, indicating safety. The 1% snakehead fish dry extract emulgel achieved the most effective burn wound healing in rabbits. The formulation is stable, safe, and has strong potential as a natural alternative for burn treatment.

Keywords: Snakehead fish; emulgel; burn wound healing; natural therapy; topical formulation

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INTRODUCTION

Burn injuries represent a significant global health concern, leading to substantial morbidity and mortality. Burn injuries cause approximately 40,000 hospitalizations and 3,400 deaths annually in the United States alone (Rowan et al. 2015). The pathophysiology of burn wounds is complex, involving immediate tissue damage, inflammatory responses, and potential systemic complications, especially in cases where the total body surface area affected exceeds 20% (Rowan et al. 2015). Effective management of burn wounds is crucial to prevent infections, promote healing, and minimize scarring.

Topical agents play a pivotal role in burn wound care, serving functions such as infection prevention, moisture retention, and facilitation of tissue regeneration. Traditional treatments have included silver sulfadiazine and various antimicrobial creams; however, these can have limitations, including delayed wound healing, cytotoxicity to keratinocytes and fibroblasts, hypersensitivity reactions, and potential bacterial resistance (Tenenhaus and Rennekampff 2025; Atiyeh and Hayek 2005). For instance, silver sulfadiazine has been reported to prolong wound healing time due to its adverse effect on fibroblast activity.

Consequently, there is a growing interest in exploring alternative therapies derived from natural sources that may offer enhanced efficacy with reduced side effects. Examples of such natural compounds include honey, aloe vera, Centella asiatica, and curcumin, which have shown promising effects in promoting wound healing through anti-inflammatory and antioxidant mechanisms (Ashok Kumar, Gousia, and Naveena Lavanya Latha 2013; Abdul-Nasir-Deen et al. 2020). This trend reflects a paradigm shift toward biocompatible, cost-effective, and eco-friendly wound care products.

One such natural source is the snakehead fish (*Ophiocephalus striatus*), indigenous to Southeast Asia. Traditionally, this fish has been consumed for its purported health benefits, particularly in wound healing. Studies have indicated that extracts from *O. striatus* are rich in bioactive compounds, including amino acids and fatty acids, which may contribute to tissue repair and anti-inflammatory effects (Sahid et al. 2018). A randomized clinical trial by Sahid et al. (2018), demonstrated that patients treated with snakehead fish extract recovered faster from surgical wounds compared to controls. In addition to the evidence mentioned, other studies have also reported the positive effects of snakehead fish extract on wound healing, such as increased tensile strength of wounds, accelerated epithelialization, and enhanced angiogenesis (Hidayati et al. 2018; Kwan, Abdul Aziz, and Ismail 2019).

Emulgels, which combine the properties of emulsions and gels, have emerged as a promising delivery system for topical applications. They offer advantages such as improved drug release, enhanced stability, and better patient compliance due to their favorable rheological properties. Moreover, emulgels are particularly suitable for incorporating hydrophobic natural extracts like snakehead fish, offering better solubility, uniform distribution, and longer retention on the wound site (Patel, Kuchekar, and Pawar 2021).

Despite the traditional use of *O. striatus* in promoting wound healing, scientific validation of its efficacy and safety, particularly in standardized topical formulations like emulgels, remains limited. Previous studies have primarily focused on oral consumption or crude extracts, with insufficient attention to controlled, topical applications. Moreover, while some research has explored the formulation of snakehead fish extract into nanoemulgels, comprehensive in vivo assessments of these formulations on burn wound healing are scarce (Tungadi et al. 2018). Additionally, the dry extract form offers specific advantages over crude or aqueous extracts, including higher stability, better preservation of active constituents, and ease of standardization for dosage formulation (Müller et al. 2011).

This gap in the literature underscores the need for rigorous scientific investigation to evaluate the therapeutic potential and safety profile of emulgels containing dry extract of snakehead fish for burn wound treatment. Addressing these gaps is essential to substantiate the traditional claims associated with *O. striatus* and to develop a scientifically validated, safe, and effective topical treatment for burn wounds.

By achieving these objectives, the study seeks to provide a scientific basis for the use of *O. striatus* extract in burn wound management and to contribute to the development of alternative therapeutic options that are both effective and safe.

The outcomes of this research hold significant implications for both clinical practice and the broader field of wound care. Demonstrating the efficacy and safety of a snakehead fish extract-based emulgel could lead to the development of a novel, natural-origin topical treatment for burn wounds, offering an alternative to conventional synthetic agents. This aligns with the increasing demand for natural and sustainable healthcare solutions.

Furthermore, this study will contribute to the scientific understanding of the wound-healing properties of *O. striatus*, potentially opening avenues for its application in other dermatological conditions. The formulation development process and methodological approaches employed can serve as a reference for future research endeavors aiming to explore natural extracts in topical therapies.

MATERIALS AND METHODS

Materials

All materials used in this study were of pharmaceutical or analytical grade, obtained from reputable suppliers: ethanol 70% (analytical grade, Merck, Germany), Hydroxypropyl Methylcellulose 22000 (pharmaceutical grade, Sigma-Aldrich, USA), distilled water (Bratachem, Indonesia), Polysorbate 80/Tween 80 (analytical grade, Sigma-Aldrich, USA), olive oil (pharmaceutical grade, Bertolli, Italy), Polyethylene Glycol 400 (analytical grade, Sigma-Aldrich, USA), methylparaben (pharmaceutical grade, Sigma-Aldrich, USA), propylene glycol (pharmaceutical grade, Sigma-Aldrich, USA), and Triethanolamine (analytical grade, Sigma-Aldrich, USA). Snakehead fish (*Ophiocephalus striatus*) dry extract were gained from PT. Royal MedicalinkPharmalab.

The following instruments were used: Hot air oven (Memmert UN55, Germany), rotary evaporator (HeidolphLaborota 4000, Germany), lyophilizer (LabconcoFreeZone, USA), digital pH meter (Hanna Instruments HI2211, USA), Brookfield viscometer (DV-E, USA), UV–Visible spectrophotometer (Shimadzu UV-1800, Japan), vortex mixer (Thermo Scientific Maxi Mix II, USA), analytical balance (Shimadzu, Japan), refrigerator (Panasonic NR-BL347, Japan), and homogenizer (Thinky homogenizer, Japan).

Methods

Preparation of Snakehead Fish Dry Extract

Fresh fish were cleaned, filleted, and dried in a hot air oven at 40°C until achieving a moisture content below 10%. The dried fish were milled into powder and extracted with 70% ethanol (1:10 w/v) by maceration for 72 h with continuous stirring at 300 rpm. The extract was filtered, concentrated under reduced pressure at 40°C using a rotary evaporator, and lyophilized to yield a stable dry powder.

Formulation of Emulgel

Three emulgel formulations containing snakehead fish dry extract at 0.25%, 0.5%, and 1% w/w were prepared. Hydroxypropyl Methylcellulose (2% w/w) was dispersed in distilled water at 2000 rpm for 2 min to obtain the gel base. The snakehead fish dry extract was dissolved in the aqueous phase. The oil phase comprising olive oil (5% w/w), Polysorbate 80 (32.5% w/w), and Polyethylene Glycol 400 (27.5% w/w) was heated to 70°C and homogenized at 300 rpm for 15 min. The oil phase was slowly incorporated into the aqueous phase under continuous stirring at 300 rpm for 15 min to form a uniform emulsion, which was then mixed with the gel base at 2000 rpm for 2 min using a Thinky homogenizer to obtain the final emulgel.

Characterization of Emulgel

Organoleptic evaluation assessed color, odor, and consistency, performed by a three-member trained panel using a 5-point scale (1 = very poor, 5 = excellent). pH was measured with a Hanna HI2211 digital pH meter by immersing the electrode directly into the emulgel at room temperature. Viscosity was measured using a Brookfield DV-E viscometer (spindle no. 6, 50 rpm). Spreadability was evaluated by placing 0.5 g of emulgel between two glass plates and measuring the spread diameter under a 100 g weight after 1 min. Adhesion time was determined by recording the time taken for two glass plates to separate under a standard load. Stability testing was performed by storing the emulgels at 25 ± 2°C (room temperature) and 4 ± 2°C (refrigerated) for 12 weeks, observing any changes in organoleptic properties, pH, viscosity, spreadability, and adhesion time.

Protein Concentration Determination

Protein content was quantified using the Bradford method Noble and Bailey (2009), with a Shimadzu UV-1800 spectrophotometer at 595 nm. Bovine serum albumin (BSA) stock solution (1 mg/mL) was prepared, and standard solutions were made at 20, 40, 60, and 80 µg/mL. One gram of emulgel was diluted with 10 mL of deionized water and vortexed at 1500 rpm for 2 min. Samples (0.5 mL) were mixed with 2.5 mL Bradford reagent, vortexed at the same speed for 30 s, incubated at room temperature for 15 min, and absorbance was measured. Each measurement was conducted in triplicate, and mean values were used for statistical analysis.

In Vivo Study Design

The in vivo study included 15 healthy adult male rabbits (*Oryctolagus cuniculus*), each weighing 1.5–2.0 kg, acclimatized for one week. Second-degree burn wounds were induced on the dorsal area using a 100°C metal plate for 10 s under anesthesia. Rabbits were randomly divided into five groups (n = 3 per group), i.e., negative control (emulgel base), positive control (Burnazin® cream), and treatment groups: 0.25%, 0.5%, and 1% snakehead fish dry extract emulgel. Treatments were applied once daily for 14 days. Wound areas were measured on days 1, 4, 8, 12, and 16 using a transparent grid.

Statistical Analysis

Data are expressed as mean ± SD. Statistical comparisons were performed using one-way ANOVA followed by Tukey's post hoc test (SPSS v25, IBM Corp., USA). A p-value < 0.05 was considered statistically significant.

RESULTS

Preparation of Snakehead Fish Dry Extract

Fresh snakehead fish were processed into dry extract prior to formulation. The fish were cleaned, filleted, and dried at 40°C to less than 10% moisture, then ground to powder. Ethanolic maceration (70% ethanol, 1:10 w/v) was performed for 72 h with continuous stirring, followed by filtration, rotary evaporation, and freeze-drying to yield a light-brown, fine powder with a characteristic odor (Figure 1).

Figure 1. Appearance of snakehead fish dry extract

Emulgel Formulation Details

Three emulgel formulations (F1, F2, F3) containing 0.25%, 0.5%, and 1% w/w of dry extract, respectively, were prepared. The detailed composition is shown in Table 1.

Table 1. Composition of snakehead fish extract emulgel formulations

Ingredient	F1 (%)	F2 (%)	F3 (%)
Snakehead fish dry extract	0.25	0.5	1.0
HPMC	2.0	2.0	2.0
Olive oil	5.0	5.0	5.0
Polysorbate 80	32.5	32.5	32.5
PEG400	27.5	27.5	27.5
Methylparaben	0.2	0.2	0.2
Propylene glycol	10.0	10.0	10.0

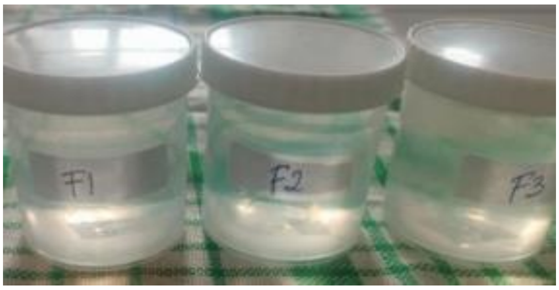

Figure 2. Visual appearance of emulgel formulations F1, F2, and F3

Physicochemical Characteristics

All three formulations were physically stable for 12 weeks at both room temperature (25 ± 2°C) and refrigerated conditions (4 ± 2°C). Measurements were performed in triplicate (n=3) and expressed as mean ± SD.

Table 2. Physicochemical parameters of emulgel formulations (mean ± SD, n=3)

Formula	pH		Viscosity(Cps)		Spreadability (cm^2)		AdhesionTime (second)	
	W_0	W_{12}	W_0	W_{12}	W_0	W_{12}	W_0	W_{12}
F10.25%	6.5±0.1	6.2±0.3	10300±50	9850±65	6.8±0.1	6.6±0.3	14.55±0.5	15.25±0.4
F20.5%	6.3±0.2	6.0±0.1	15640±60	15100±70	6.9±0.2	6.7±0.2	15.51±0.6	15.05±0.3
F31%	6.2±0.1	6.2±0.2	19540±55	19440±50	6.9±0.1	6.8±0.1	14.45±0.7	15.55±0.2

Protein Content

Protein content increased proportionally with extract concentration. Results are mean ± SD of triplicate measurements.

Table 3. Protein content of snakehead fish extract emulgel (mean ± SD, n=3)

Formula	Protein content (%) ± SD
F10.25%	7.56 ± 0.04
F20.5%	8.58 ± 0.05
F31%	9.11 ± 0.06

In Vivo Burn Wound Healing

Figure 3 shows representative images of burn wounds in each group on days 0, 4, 8, 12, and 16. The F3 group demonstrated the fastest healing, with complete wound closure by day 16.

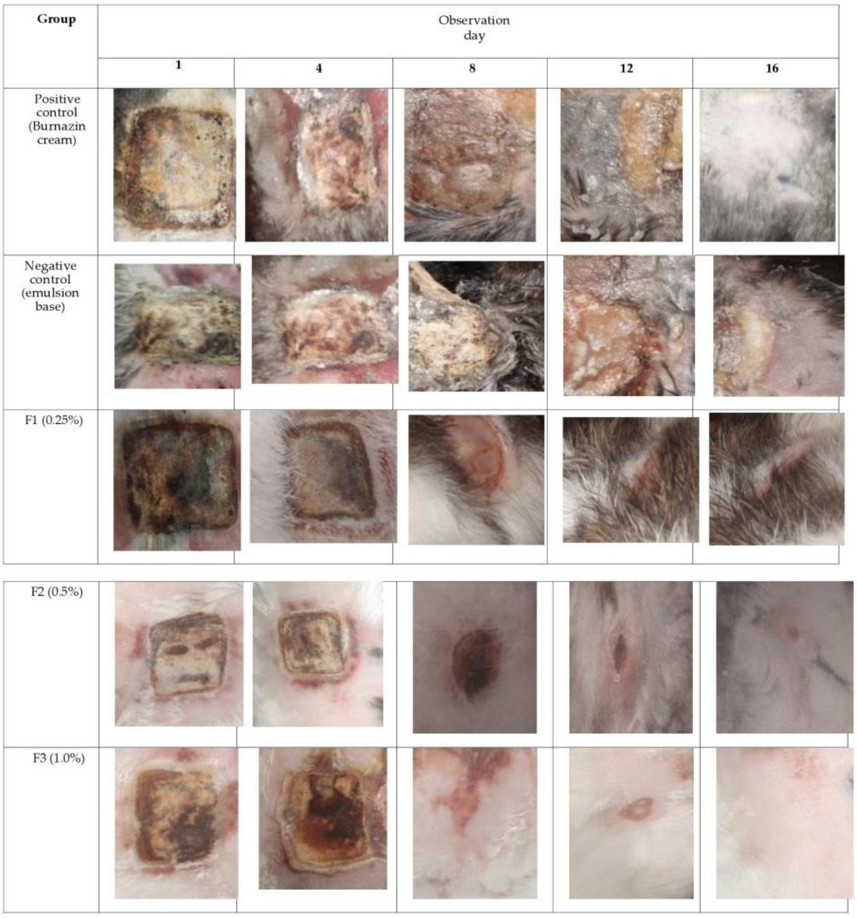

Figure 3. Representative wound images in rabbits treated with different formulations over 16 days

Quantitative wound area measurements (mean ± SD, n=3 per group) are presented in Table 4.

Table 4. Wound area reduction over time (cm^2, mean ± SD)

Group	Replication	Day to 0 (cm^2)	4 (cm^2)	8 (cm^2)	12 (cm^2)	16 (cm^2)
KP	1	1.5±0.0	1.5±0.0	1.0±0.1	0.4±0.03	0±0.0
	2	1.5±0.0	1.5±0.0	0.9±0.1	0.25±0.03	0±0.0
	3	1.5±0.0	1.5±0.0	1.0±0.1	0.2±0.03	0±0.0
	Average	1.50±0.0	1.50±0.0	0.96±0.04	0.28±0.03	0.00±0.0
KN	1	1.5±0.0	1.5±0.0	1.3±0.04	1±0.05	0.3±0.02
	2	1.5±0.0	1.5±0.0	1.4±0.04	0.8±0.05	0.2±0.02
	3	1.5±0.0	1.5±0.0	1.2±0.04	0.6±0.05	0.15±0.02
	Average	1.50±0.0	1.50±0.0	1.30±0.04	0.80±0.05	0.22±0.02
F1(0.25%)	1	1.5±0.0	1.5±0.0	1.1±0.06	0.7±0.04	0.2±0.02
	2	1.5±0.0	1.5±0.0	1±0.06	0.5±0.04	0.09±0.02
	3	1.5±0.0	1.5±0.0	1±0.06	0.4±0.04	0.02±0.02
	Average	1.50±0.0	1.50±0.0	1.03±0.06	0.53±0.04	0.10±0.02

F2 (0.5%)	1	1.5±0.0	1.5±0.0	0.8±0.05	0.6±0.03	0.01±0.0
	2	1.5±0.0	1.5±0.0	0.6±0.05	0.4±0.03	0±0.0
	3	1.5±0.0	1.5±0.0	0.6±0.05	0.3±0.03	0±0.0
	Average	1.50±0.0	1.50±0.0	0.66±0.05	0.43±0.03	0.00±0.0
F3 (1%)	1	1.5±0.0	1.5±0.0	0.8±0.05	0.5±0.03	0±0.0
	2	1.5±0.0	1.5±0.0	0.7±0.05	0.3±0.03	0±0.0
	3	1.5±0.0	1.5±0.0	0.5±0.05	0.2±0.03	0±0.0
	Average	1.50±0.0	1.50±0.0	0.67±0.05	0.33±0.03	0.00±0.0

KP : Positive control (Burnazin® cream)
KN: Negative control (emulgelBase)

DISCUSSION

The present study evaluated both efficacy and safety of snakehead fish (*Ophiocephalusstriatus*) dry extract emulgel in the treatment of burn wounds. The findings demonstrated that all formulations, particularly F3 (1% w/w snakehead fish dry extract emulsion), promoted wound healing comparable to Burnazin® cream, with no visible signs of irritation or adverse local reactions, indicating favorable topical safety. These observations align with previous studies demonstrating the wound-healing properties of *Channa striata* extract due to its high protein, amino acid, and fatty acid content (Kadir et al. 2022).

Burn wounds undergo a complex healing process, involving hemostasis, inflammation, proliferation, and remodeling phases (Jeschke et al. 2015). The photographic evidence from this study suggests that wounds treated with 1% SFDE in emulsion formulation exhibited faster re-epithelialization, reduced necrotic tissue, and improved scar formation by day 16 compared to the control groups (Figure 3). This outcome is likely attributed to the presence of bioactive compounds such as glycine and polyunsaturated fatty acids in *Channa striata*, which have been reported to accelerate fibroblast proliferation and collagen synthesis, essential for tissue regeneration (Ali et al. 2018).

In contrast, the negative control group displayed delayed wound healing, characterized by persistent inflammation and incomplete tissue remodeling. The positive control (Burnazin cream) demonstrated moderate healing effects, but the snakehead fish extract emulgel outperformed it in terms of tissue regeneration and scar maturation. The efficacy of snakehead fish extract in accelerating wound healing has been corroborated by previous research, which highlighted its role in upregulating growth factors such as transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF), both crucial for angiogenesis and granulation tissue formation (Taslim et al. 2022). This can be seen in figure 4.

Moreover, the emulgel formulation used as a vehicle for the active compound may have contributed to the enhanced healing effects by improving the bioavailability and penetration of the extract into the deeper layers of the wound (Choudhury et al. 2017). Emulgels have been widely recognized as effective drug delivery systems due to their ability to encapsulate hydrophobic bioactive compounds, thereby enhancing their therapeutic potential in wound healing applications (Levin et al. 2022).

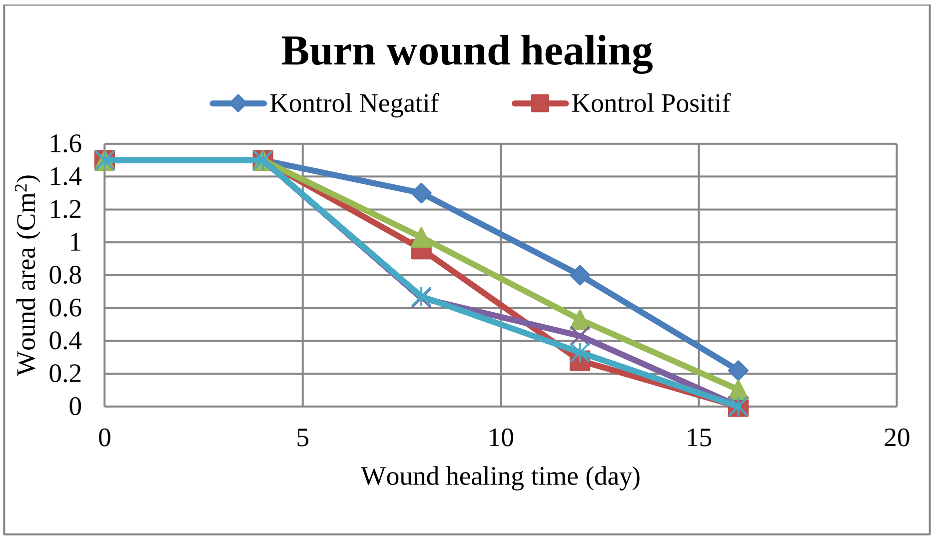

Figure 4. Burn wound healing curve

The preparation of dry extract from *O.striatus* involved low-temperature drying (40°C) and lyophilization, processes that help preserve thermolabile bioactive compounds such as albumin, essential amino acids, and fatty acids, which are known to support tissue regeneration (Kwan, Aziz, and Ismail 2020). Compared to crude or aqueous extracts, dry extracts offer improved stability, reduced microbial load, and ease of incorporation into topical formulations (Ibrahim et al. 2018).

Although *O. striatus*and *C. striata* are both commonly referred to as snakehead fish, their bioactive profiles are not identical. *O. striatus* is reported to have higher levels of certain amino acids (e.g., glycine, arginine, and glutamine) and omega-3 fatty acids, while *C. striata* may exhibit different peptide compositions (Zheng, Yang, and Ba 2025). These compositional differences are important, as they may influence the wound-healing mechanism, such as collagen synthesis, angiogenesis, and anti-inflammatory activity, supporting the choice to specifically investigate *O. striatus* extract in this formulation.

Physicochemical characteristics are critical for the performance and suitability of topical products in burn management. The pH values of all emulgel formulations (6.19–6.50) fall within the skin's natural range (4.5–6.5), minimizing the risk of irritation and supporting the healing microenvironment (Proksch 2018). Viscosity influences drug release and retention at the application site; the moderate viscosities observed ensure prolonged contact with the wound while still allowing for easy spreadability. Spreadability directly affects patient comfort and ease of application, especially important in burn patients with tender skin, while also influencing the uniformity of drug delivery over the wound surface. Adequate adhesion time helps maintain the formulation on the wound, protecting it from contamination and desiccation, which are key factors for optimal healing (Boateng and Catanzano 2015). These attributes, collectively, ensure the formulation's therapeutic effectiveness and patient compliance.

The in vivo results showed that F3 achieved complete wound closure by day 16, suggesting that the higher concentration of *O. striatus* dry extract may enhance fibroblast proliferation, collagen deposition, and epithelialization. No erythema or edema was observed throughout the study period, supporting the formulation's topical safety. The combination of favorable physicochemical properties, bioactive-rich *O. striatus* dry extract, and demonstrated in vivo wound healing activity underscores the potential of this emulgel as an effective and safe topical therapy for burn wounds.

CONCLUSION

This study successfully developed and evaluated an emulgel formulation containing dry extract of snakehead fish (*Ophiocephalus striatus*) for burn wound treatment. The formulation demonstrated favorable physicochemical characteristics, good topical safety, and significant wound healing efficacy, with the 1% formulation showing optimal results comparable to Burnazin®. In conclusion, the findings confirm that *O. striatus* dry extract emulgel has potential as a safe and effective topical therapy for burn wounds. Future studies are recommended to further explore its mechanism of action, conduct long-term safety assessments, and evaluate clinical efficacy in human subjects.

ETHICAL ISSUES

All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee of State University of Gorontalo with recommendation number 70/UN47.B7/KE/2023.

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