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Formulation and Evaluation of Snakehead Fish Mucus Emulgel for Enhanced Wound Healing and Antibacterial Activity

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Abstract: The snakehead fish (Channa striata) is rich in protein, including albumin, which is beneficial for health by aiding in tissue formation, wound healing, and maintaining fluid balance in cells. In addition to the meat, the fish mucus also contains a substance that is effective for wound healing and has antibacterial properties. This study aimed to formulate and assess the snakehead fish mucus emulsion for its wound healing and antibacterial activity. Emulgel formulations were made with varying concentrations of snakehead fish mucus: formula 1 (5%), formula 2 (10%), and formula 3 (15%). The evaluation of the emulgel included organoleptic, pH, moisture, homogeneity, and sensitivity tests. The wound healing experiment involved 15 test animals, divided into 5 groups, each consisting of three rats. The emulgel's antibacterial activity was tested against Staphylococcus aureus bacteria. The data obtained was analyzed using SPSS with the one-way ANOVA method. The study found that snakehead fish mucus emulgel showed a white color, emulgel, typical smell, pH of 5, homogeneity, and spreadability of 5.5 – 6.6 cm. The ability of snakehead fish mucus emulgel exhibited wound closure on day 5 by formula 3, followed by formulas 2 and 1. The potential of formula 3 as a wound-healing was comparable to the positive control. Data analysis showed that the antibacterial activity against Staphylococcus aureus was strongest at a concentration of 15%, with a zone of inhibition of 10.25 mm. The study concludes that snakehead fish mucus can be formulated into an emulgel and has antibacterial activity for wound healing in hyperglycemic rats.

Keywords: antibacterial; emulgel; *Staphylococcus aureus*; wound healing.

1. Introduction

The snakehead fish (Channa striata) is a freshwater species indigenous to the water systems of Indonesia, particularly prevalent in Sumatra, Java, and Kalimantan. Noted for its substantial protein and mineral content, this fish offers notable health benefits. Commonly, snakehead fish are subjected to processing methods that result in the production of crackers, pempek, processed dried fish, and various other prepared dishes [1]. The snakehead fish has been associated with potential medicinal properties, as its components have been clinically proven to aid in treating various conditions, such as wound healing [2]. In addition to fish flesh, a previous study has proven that fish mucus is a highly effective ingredient for multiple therapeutic applications, exhibiting activities similar to those of fish meat [3].

Fish mucus contains many biologically active compounds that are constitutively expressed to provide immediate protection to fish from potential pathogenic microbes and parasites. Epidermal mucus is produced by mucus cells, predominantly composed of water and gel macromolecules, such as mucins and other glycoproteins. The composition of mucus secretions can vary based on environmental factors, such as hyperosmolarity and pH levels between 9 and 11. These compounds include lysozyme, lectins, proteolytic enzymes, flavoenzymes, immunoglobulins, C-reactive protein, apolipoprotein A-I, and antimicrobial peptides [4-6]. The mucus layer on the fish's surface serves various essential functions, including disease resistance, respiration, regulation, communication, feeding, and nest building. It has been reported that removing epidermal mucus and exposing fish to a pathogenic bacterium causes an increase in mortality in several fish species [7].

Any injury to the fish's skin can lead to infection, which can be deadly to the fish. Mucus is secreted to act as a barrier against disease, and it is known to have antibacterial activity. Therefore, the complex components of fish mucus contribute to some pharmacological activity, including wound healing and antibacterial activity [8,9].

In the wound healing process, fibroblast growth factors (FGFs) are growth factors that can directly regulate cell proliferation, migration, and differentiation [10-12]. The previous study exhibited that arginine in the fish mucus is the precursor of nitric oxide, contributing to the immune response, angiogenesis, epithelialization, and tissue formation in wound healing [13,14]. Thus, we believe that the wound-healing impact of snakehead fish mucus can be significantly improved by administering it in an effective dosage form, such as an emulgel.

Emulgels are a novel and promising drug delivery technology for administering hydrophobic medicines [15,16]. This formulation combines emulsion and gel, making it a new drug delivery mechanism. Emulgel consists of two phases of organic molecules that penetrate water as a gel and a small phase of oil emulsion. The presence of an oil phase makes emulgel superior to gels, which stick to the drug for a long time on the skin, have good dispersion, are easy to apply, and provide comfort to the skin [17,18]. By considering the advantages of emulgel, we aim to formulate an emulgel containing snakehead fish mucus and then evaluate its activity in wound healing and its antibacterial activity.

A previous study has indicated that the application of an emulgel containing snakehead fish extract can expedite the process of wound healing. This is evidenced by a reduction in wound length, an increase in the quantity of neutrophil and macrophage cells, a rise in the average number of fibroblasts, and a higher collagen density in white mice [19]. However, it is worth noting that the study utilized a mucus concentration of only 10%, leaving the optimal concentration for promoting wound healing unknown. Our previous study using a patch formula derived from snakehead fish mucus indicated that a 5% concentration of the mucus accelerated the wound healing process in diabetic rats [20].

The skin mucus extract of snakehead fish showed a powerful ability to inhibit the growth of Escherichia coli. It also caused significant damage to the cell structure and led to a high rate of cell mortality. In addition, the analysis of metabolic pathways revealed disruptions in several pathways, including pyrimidine and purine metabolism [21]. Thus, the study aims to develop emulgel preparations using various concentrations of snakehead fish mucus and evaluate the wound-healing and antibacterial activities.

Here, we formulated the emulgel containing the snakehead fish mucus in various concentrations: 5, 10, and 15%. The emulgel was evaluated for its characteristics, wound-healing effect, and antibacterial activity. In the wound healing evaluation, we used a group of mice; besides the antimicrobial activity, we conducted it on the pathogenic bacteria Staphylococcus aureus.

2. Materials and Methods

2.1. Chemicals.

Analytical grade was used for all solvents, reagents, and standards. Deionized water was obtained through a Millipore-Q50 Ultrapure water system (Sartorius, USA). Alloxan monohydrate (Merck Pte. Ltd., Singapore), Vipalbumin®, biuret reagent, emulsifier water phase, and oil phase were obtained commercially in Makassar. The positive control used in the antibacterial evaluation was amoxicillin (Merck Pte. Ltd., Singapore). The mucus of snakehead fish (Aquaculture), medium nutrient agar (Merck Pte. Ltd., Singapore).

2.2. Instrumentation.

A UV-Vis spectrophotometer (LAMDA 365, PerkinElmer Inc., USA) was utilized as the analytical equipment. In the process of lyophilization, a vacuum freeze-drier (LGJ 10, Armfield Limited, UK) was used. Every piece of glassware used was of analytical grade.

2.3. Sample preparation.

Skin mucus was prepared according to the previous study with minor modifications [20]. The snakehead fish were caught in Makassar, Indonesia. The fresh snakehead fish were cleaned using clean water and placed in a stainless steel container. Skin evaporation was used to harvest the fish mucus. The mucus was steamed at 40°C-50°C until an extract formed, and then the corpus was freeze-dried.

2.4. Formulation of emulgel.

Emulgel was prepared according to the previous study with slight modifications [22]. Emulgel was produced in quantities of as much as 100 g using the emulsifier water phase (sodium benzoate, glycerin, Tween 80, fish mucus) and the oil phase (Span 80, cetyl alcohol, stearic acid, and liquid paraffin). The temperature of each stage is raised to 70 °C. Finally, the emulsified mixture is homogenized by intermittent shaking to produce a translucent emulgel, and the oil phase is added to the water phase to produce the emulsion [22]. Emulgel formulations are distinguished by the concentration of the active ingredient in the form of mucus extract. This includes formulations 1, 2, and 3 with mucus extract contents of 5%, 10%, and 15%, respectively.

2.5. Evaluation of physicochemical properties of emulgel.

The qualities of the produced emulgel were assessed based on its color, scent, and texture. The pH of the emulgel was measured at room temperature by dissolving 1 g of the emulgel in 10 mL of distilled water, stirring, and using pH indicator paper to determine the pH.

To check for homogeneity, a sample of the emulgel was spread on a transparent glass, and it was expected to appear uniform with no visible coarse grains. The spreadability test involved placing 1 g of emulgel on a glass slide, covering it with another slide, applying a 50 g weight for 1 minute, and then measuring the spread diameter [22].

2.6. Animals.

The mice were obtained commercially and kept in a cage with access to food and water. The temperature (23–25°C) was maintained via a light/dark cycle of 12 hours [23]. The mice were divided into five groups of three: positive control, negative control, and treatment with formulas 1, 2, and 3, making a total of 15 mice used in the study. To assess the healing rate of diabetic ulcers in mice, we conducted eight days of wound surveillance. The animal studies were carried out in compliance with the laboratory regulations for animal use at Universitas Megarezky, Makassar, Indonesia (No: 008.C/07.091056/ X/2021).

2.7. Alloxan-induced diabetic model.

Alloxan monohydrate was weighed and mixed with 0.2 mL of saline for each animal based on its weight. The alloxan was injected into the peritoneum at a dosage of 150 mg/kg body weight. After administering the alloxan, the animals were allowed to eat, and a 5% dextrose solution was provided to counteract the hyperglycemic phase. After 48 hours, the animal's blood sugar levels were measured using a glucometer. Rats with high blood sugar levels (glucose level >300 mg/dL) were isolated and divided into five groups, with three mice in each group [24].

2.8. Evaluation of wound healing activity.

Randomly, the diabetic mice were divided into five groups. The mice were burned on the right upper thigh, and the wound was 2 cm long. The mice in group I served as a control, while the mice in group II were treated with Vipalbumin® as a positive control. The mice in groups III, IV, and V were given an emulgel formula 1, 2, and 3, respectively. After 14 days of treatment, wound healing procedures were conducted [20].

2.9. Evaluation of antibacterial activity.

The antibacterial activity was determined using a modified version of the agar disc diffusion method described in a previous study [25]. A single colony of *Staphylococcus aureus* was prepared as a suspension in 0.85% sodium chloride (NaCl) solution to adjust the turbidity of the bacterial suspension with an approximate cell count density of $1.0 \times 10^{\wedge 8}$ colony-forming units per milliliter (CFU/mL). Using a swab, the inoculum (1.0×10^8 CFU/mL) was spread in three directions on the surface of nutrient agar. Sterile filter paper discs of 6 mm diameter were impregnated with 5%, 10%, and 15% emulgel. The discs were placed on the surface of the nutrient agar and incubated for 24 hours at 37°C. The antimicrobial activity was evaluated by measuring the diameter of the inhibition zone (in millimeters ± standard deviation). The experiments were conducted in triplicate. Bacterial colonies that showed inhibition zones smaller than 8 mm were considered insensitive to the emulgel [25]. The positive and negative controls used in this study were amoxicillin and emulgel, respectively.

2.10. Statistical analysis.

The presented data are the means ± SD of at least three independent experiments. Statistical analysis was performed by GraphPad Prism 8.0. Differences between groups were analyzed by one-way ANOVA followed by Tukey's multiple-comparison test.

3. Results and Discussion

The utilization of natural resources from both plants and animals has been widespread. Moreover, utilization strategies that aim to reduce industrial waste are gaining popularity among researchers nowadays [26]. The utilization of snakehead fish [20], shrimp, and crab shell waste has been extensively examined by earlier researchers [27,28]. Our prior study indicates that snakehead fish mucus can be made into a transdermal patch, potentially affecting wound healing [20]. To determine the stability and efficacy of this mucus emulgel formulation, we prepared snakehead fish mucus into an emulgel dosage form for this investigation.

Emulgel offers several advantages over other topical dosage forms. It can increase the viscosity of the water phase, is thixotropic, non-greasy, easy to apply and remove, emollient, non-staining, and has a transparent and attractive appearance. It is also environmentally friendly [29]. Snakehead fish mucus extract is used to create an emulgel due to limitations in delivering hydrophobic drugs. This stable emulsion and cream formula helps reduce the phase interfacial tension, enabling the delivery of snakehead fish mucus extract and penetration of lipid membranes. Previous studies have shown that a mucus extract-containing emulgel formulation could be utilized for treating wound healing [30]. However, further fundamental evaluation needs to be elaborated.

3.1. Evaluation of physicochemical properties of emulgel.

This study was conducted on snakehead fish mucus that was crystallized and used in the preparation of emulgel in three different formulas. Each formula contains different quantities of mucus: formulas 1, 2, and 3 contain snakehead fish mucus at 5%, 10%, and 15%, respectively. The organoleptic test results of the emulgel formulas showed positive characteristics, as detailed in Table 1.

Table 1. Characteristic of emulgel containing snakehead fish mucus.

Emulgel	Observation					
	Color	**Smell**	**Consistency**	**pH**	**Homogeneity**	**Spreadability**
Formula 1	White	Typical	Thick	5	Homogeneous	5.5 cm
Formula 2	White	Typical	Thick	5	Homogeneous	6.6 cm
Formula 3	White	Typical	Thick	5	Homogeneous	6.6 cm

The pH plays a crucial role in the effective delivery, stability, and compatibility of topical formulations for human skin [31,32]. Human skin typically has a pH ranging from 4.5 to 6.5, and it is essential that the pH of the formulation falls within this range to prevent potential irritation [33]. Our produced emulgel has undergone pH testing and has been found to be within the optimal pH range. Furthermore, the homogeneity test results presented in Table 1 indicate the absence of visible coarse grains in the emulgel preparations, confirming their uniformity. Additionally, as indicated by the Directorate General of POM, the absence of visible coarse grains is an indicator of the emulgel's high quality [34].

3.2. Evaluation of wound healing activity.

After conducting a one-way ANOVA to analyze the impact of snakehead fish mucus extract formulation in emulgel formula on the healing of diabetic mellitus wounds, it was found that there was no significant difference in wound closure between formula 3 and the positive control, as indicated in Table 2. On the fifth day, all the wounds on the rats had healed, regardless of whether they were treated with formula 3 or the positive control. The healing rate of formulas 1 and 2 was slower than that of the positive control, with wound closure observed on the seventh day. Conversely, even 14 days after treatment, there was no wound closure in the negative control group. The use of snakehead fish mucus in emulgel formulation was shown to have a positive healing effect by promoting the generation of fibroblasts.

Table 2. Observations on the treatment of wounds with snakehead fish mucus emulgel.

Treatment	Observation (cm)				
	Control (-)	**Control (+)**	**Formula 1**	**Formula 2**	**Formula 3**
Day 1	2.0	1.8	1.3	1.5	1.6
	2.0	1.7	1.5	1.5	1.6
	1.7	1.8	1.8	1.5	1.5
Day 3	1.2	1.5	1.0	1.3	1.0
	1.4	1.5	1.0	0.9	1.3
	1.4	1.5	1.2	1.0	1.2
Day 5	1.0	0	0.7	0.5	0
	1.0	0	0.5	0.9	0
	1.0	0	1.0	0.5	0
Day 7	0.9	0	0	0	0
	0.9	0	0	0	0
	0.9	0	0	0	0
Day 14	0.5	0	0	0	0
	0.4	0	0	0	0
	0.7	0	0	0	0

A previous study exhibited that the optimal level of wound closure from emulgel containing the extract mucus of snakehead fish occurred on day 7, with a positive control of 10% povidone-iodine [19]. Meanwhile, this research shows that on day 5, the ability to close the wound optimally occurs in alloxan-induced hyperglycemic rats with the same ability as the positive control, which is a commercial albumin preparation [35].

3.3. Evaluation of antibacterial activity.

Snakehead fish mucus in emulgel has an antibacterial effect characterized by forming an inhibition zone against *Staphylococcus aureus*, as shown in Table 3. The positive control, amoxicillin, and formula 3 showed strong antibacterial activity. The bactericidal activity of the other formulations, 1 and 2 formulas, was moderate. The presence of an inhibition zone caused by the emulgel can be attributed to the compounds found in the mucus extract of the snakehead fish (Figure 1). The mucus secreted through the epidermis of snakehead fish contains numerous antibacterial peptides that protect the fish from pathogens [36,37]. These antibacterial peptides denature bacterial cell proteins and damage their cytoplasmic membrane [21]. Damage to the cytoplasmic membrane by antibacterial peptide compounds can lead to the leakage of essential metabolites and the deactivation of bacterial enzyme systems [38]. This situation can cause bacterial death by allowing nucleotides and amino acids to escape and by preventing the entry of active ingredients into the cell [21].

Antimicrobial peptides extracted from the skin mucus of aquatic organisms definitively exhibit potent inhibitory effects on a wide range of bacteria, such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Vibrio harveyi* [39-41]. Moreover, natural immune-related elements such as lectin, immunoglobulin, and lysozyme have been noted to play an equally vital role in protecting against pathogen infiltration [42].

Table 3. Antibacterial activity of emulgel containing snakehead fish mucus toward *Staphylococcus aureus.*

Emulgel	Diameter (mm)	Category
Control (-)	0	-
Control (+)	21.17	Strong
Formula 1	8.01	Moderate
Formula 2	9.09	Moderate
Formula 3	11.16	Strong

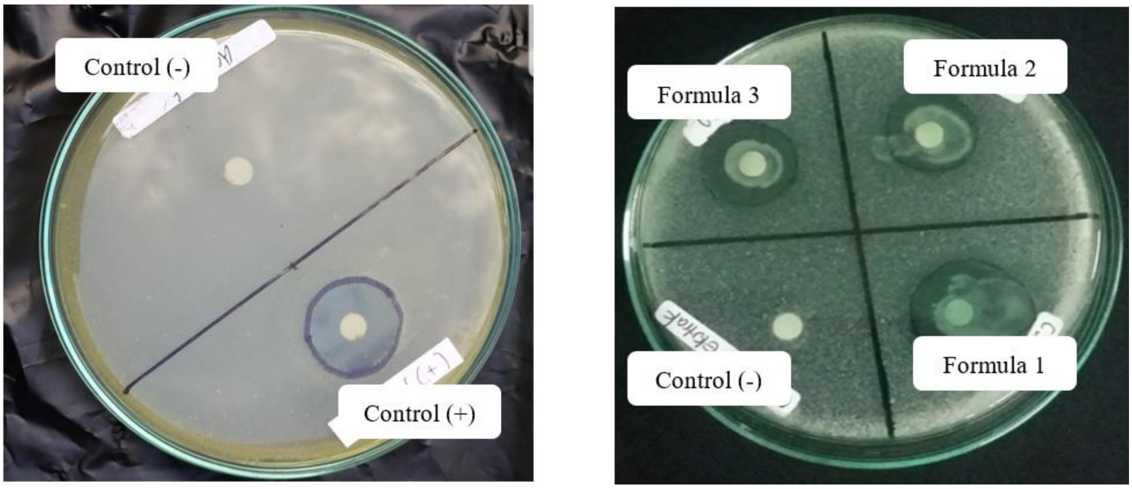

Figure 1. Diameter of the bacterial growth inhibition zone between the control and formulas.

The antibacterial activity can be categorized based on the diameter of the bacterial growth inhibition zone [43]. Antibacterial activity is classified as strong, moderate, or weak with inhibition zone diameters of >6 mm, 3-6 mm, and <3 mm, respectively [44].

4. Conclusions

Formulation of an emulgel containing the mucus of the snakehead fish was accomplished with adequate characteristics. The wound healing test revealed that the emulgel of three different concentrations of snakehead fish mucus accelerated wound healing in hyperglycemic rats. In addition, emulgel containing the mucus of the snakehead fish has antibacterial activity against the pathogenic bacteria Staphylococcus aureus. Thus, emulgel containing mucus extract is very promising, and it is important to carry out further evaluation to complete the activity data of emulgel formulas.

Author Contributions

Conceptualization, B.Y. and S.; methodology, B.Y. and Y.Y.D.; software, M.F.; validation, B.Y., S., and M.A.M.; formal analysis, A.D.P., M.A., and M.F.; investigation, B.Y. and Y.Y.D.; resources, B.Y. and S.; data curation, A.D.P., M.A., M.A.M. and M.F.; writing—original draft preparation, B.Y. and M.F.; writing—review and editing, B.Y., S., Y.Y.D. and M.F.; visualization, B.Y. and M.F.; supervision, S. and Y.Y.D.; project administration, B.Y. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

The animal study protocol was approved by the Institutional Ethics Committee of Universitas Megarezky (No: 008.C/07.091056/X/2021 in October 2021).

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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