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RESEARCH ARTICLE

Therapeutic Potential of Chinese Edible Frog (*Hoplobatrachuschinensis)* Powdered Skin for Enhanced Wound epithelialization and Rapid Wound Recovery

Renwel John B. Lacaden[1], Ivie C. Seangoy[2], Analiza L. Marcos[3], Jonathan P. Pasio[4]

[1,2,3,4]Nueva Vizcaya State University"

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***Corresponding Author:**

jppasion@nvsu.edu.ph

ABSTRACT

Wound healing is a complex process crucial for tissue restoration and functionality. Despite advancements in medical field, challenges persist with the use of conventional approaches in wound healing, particularly with antibiotic resistance pathogen.

This study investigated the presence of bioactive compounds, particularly peptides, in *Hoplobatrachuschinensis* frog skin. A Color test using Ninhydrin and Biuret was conducted which confirmed the presence of peptides in the powdered frog skin, indicating the skin's potential therapeutic properties. The study further examined the effect of different concentrations of powdered frog skin on wound contraction rate, epithelialization time, and overall healing.

Statistical analysis showed that the skin of *H.chinensis* accelerated the wound recovery time, wound epithelialization and increases wound contraction rate. Results revealed that higher concentrations of powdered frog skin led to faster wound contraction and epithelialization, as well as shorter overall healing times.

In conclusion, *Hoplobatrachuschinensis s*kin contained bioactive compounds that can promote wound healing, with higher concentrations of powdered frog skin shows superior therapeutic effects. These findings suggest that powdered frog skin could serve as a promising natural alternative for wound management.

INTRODUCTION

Wound healing is a complex and vital physiological process characterized by a sequence of intricate biological events that culminate in the restoration of tissue integrity and functionality (Falanga, 2005; Bazaz*et al.,* 2013). This dynamic reparative process hinges upon the orchestrated participation of diverse cell types, including fibroblasts, inflammatory cells, endothelial cells, and epidermal cells, which collectively contribute to the meticulous reconstruction of skin in both physical and mechanical dimensions (Gal *etal*., 2009; Mahere RezazadeBazaz*et al.,* 2013).

Modern wound healing methods have long relied on established practices, including the administration of antibiotics to combat infection and the use of various dressings to protect wounds and promote tissue regeneration (Aditya Sood*et al.,* 2014). While these conventional approaches had achieved success in numerous cases, they were not without limitations. Among these concerns was the emergence of antibiotic-resistant pathogens, an alarming global health issue that threatens the efficacy of antibiotic-based wound care (Tacconelli, 2017).

Consequently, the pursuit of novel wound healing treatments has emerged as a paramount challenge in the realm of medical science (Prakash Monika, 2022). The emerging interest in the exploration of natural remedies that may harbor augmented wound healing capabilities derived from diverse animal sources has garnered global attention (Dr. Sneha Shah, 2023). Folk medicine practitioners have sought to introduce new wound healing agents. These agents include plants and animal products with special properties for the treatment of wounds and the prevention of wound infection (Victor Wong, 2013).

Recent years have witnessed a resurgence of interest in the potential of natural and unconventional materials to address the limitations of traditional wound care. Nature, with its vast array of resources, has inspired researchers to seek alternative solutions for wound healing, and among the intriguing possibilities is the utilization of frog skin, an organism-derived material with distinctive biochemical attributes that could significantly influence the wound healing process (Purna Sai *et al.,* 2022).

Several studies have examined the bioactive compounds present in the skin secretions of *Hoplobatrachusrugulosus.* Conlon *et al.,* (2014) reported that these secretions contained a diverse array of molecules with antimicrobial, anti-inflammatory, and tissue regeneration properties. These compounds have the potential to combat infection at wound sites and facilitate the healing process (Li *et al.,* 2022). One of the primary areas of interest in frog skin secretions was their ability to accelerate the wound healing process, Chen *et al*., (2019) researched peptides isolated from frog skin and found that they can enhance wound healing by stimulating collagen synthesis and angiogenesis, these peptides may play a crucial role in promoting tissue repair and regeneration. Furthermore, Smith and Jones (2019) cast a spotlight on the bioactive compounds residing within amphibian skin secretions, evoking significant intrigue due to their potential to modulate immune responses and inflammation during the wound healing process and enhance wound contraction in the animal model.

The skin of the Chinese Edible Frog, colloquially referred to us as “tukak” in the Philippines, has evolved to serve as an effective shield against external environmental factors and microbial threats (Purna Sai *et al.,* 2022), its remarkable regenerative properties, coupled with the presence of bioactive compounds (peptides and proteins), prompt an exploration into the possibility of harnessing its therapeutic potential to expedite the process of wound recovery. Frog derived immunomodulatory peptides promoted cutaneous wound healing by regulating cellular response (Xioquin He *et al.,* 2022). It had been considered among the byproducts researched for use as biological dressing due to specific characteristics such as high collagen concentration and the presence of anti-microbial and lipid peptides (Purna Sai *et al.,* 2022). These factors optimized the healing process and created a protective barrier against opportunistic microorganisms in the wound when used as an occlusive dressing (Alves, 2022 in Santos *et al.,* 2020).

Adding depth to the exploration of frog skin's therapeutic potential was the historical use of this biological resource in traditional and indigenous wound healing practices, the traditional medicinal modality for biological wound dressing in Vietnam, Africa, and South America, had yielded promising results (Shores, 2007 in Purna Sai *et al.,* 2022). Findings pointed out that in many ancient cultures, amphibians were believed to possess medicinal properties to treat various illnesses. (Gomes, *et al.,* 2007). This underscored the imperative nature of investigating *Hoplobatrachuschinensis* powdered skin for its purported antimicrobial attributes.

In light of the preceding considerations, the present study examined the biochemical composition of frog skin, with the primary objective of identifying the presence of bioactive compounds such as peptides that could substantiate its acclaimed wound healing attributes. Furthermore, this research investigated the healing properties of frog skin and its potential to accelerate the intricate cascade of events characterizing the wound healing process. Through a comprehensive exploration of these aspects, the researchers aimed to contribute valuable insights that could potentially revolutionize the field of wound care and offer avenues for the development of effective wound healing therapeutics.

Moreover, the research aimed to uncover the therapeutic potential of *H.chinensis* skin for wound contraction and epithelialization by addressing the days for complete epithelialization and the rate of wound contraction in rats treated with various formulations of powdered *H. chinensis* skin compared to a control group receiving liquid paraffin wax. Additionally, the study analyzed the significant difference in the rate of contraction between different treatments and the controlled group.

MATERIALS AND METHODS

The researchers utilized a completely randomized design to ensure unbiased allocation of experimental units to treatment groups. This approach enhanced the validity and reliability of the research findings by minimizing systematic bias and confounding variables. Albino wistar rats were utilized as the experimental units. Each rat had an equal chance of receiving any treatment through random assignment. The study systematically investigated the effects of powdered frog skin on wound healing by establishing several treatment groups, including varying dosages. Each treatment group represent a distinct experimental condition, facilitating comparative analysis of treatment outcomes.

The methodological flowchart in Figure 1 presents systematic steps for this research study. Initial stage comprised of the establishment of animal model, specifically the rat collection and frog identification, collection, and processing to acquire the correct animal model needed. To ensure the reliability of data, a second phase was conducted employing chemical assays as confirmatory test to verify the presence of wound-healing properties. The third phase involved wound preparation and treatment application to create controlled conditions, it included anesthesiology to ensure the subjects' well-being and safety. The fourth phase comprised the measurement of wound contraction and epithelialization to gather the necessary data. The final phase involved euthanasia through the administration of sodium pentobarbital (PB) via intraperitoneal injection, adhering the ethical standards for humane euthanasia in animal research.

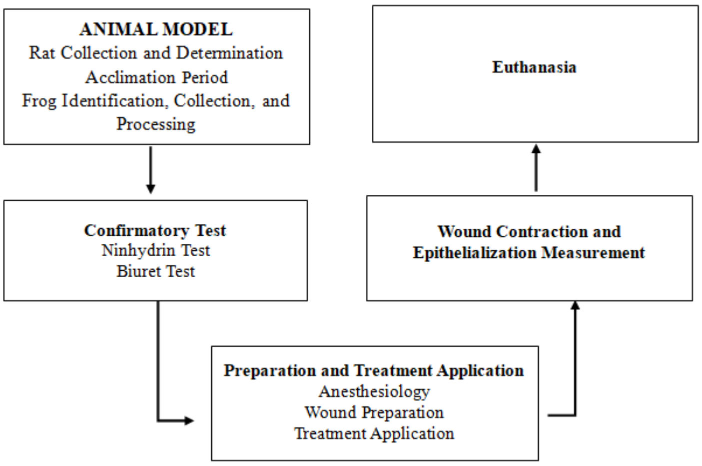

Figure 1. The Methodological Flow Chart of the Study

The Animal Model

The animal model used in this study comprised 3-4-month-old male albino Wistar rats with anticipated body weights ranging from 100 to 150 grams. These rats were purchased from C and A Pet shop, located in Bayombong, Nueva Vizcaya. Upon procurement, the animals were individually housed in separate cages to ensure their welfare and prevent potential cross-contamination. The rats were maintained under controlled conditions with access to a standard diet and water ad libitum.

The rats were randomly divided into four groups (n=4) to ensure statistical validity and minimize bias, The Control group received 0.5 ml of liquid paraffin wax. Treatment 1 involved treating the rats with a combination of 0.002 grams of powdered frog skin (PFS) and 0.5 ml of liquid paraffin wax. Treatment 2 involved treating the rats with 0.006 grams of PFS and 0.5 ml of liquid paraffin wax. Treatment 3 involved treating the rats with a combination of 0.010 grams of PFS and 0.5 ml of paraffin wax.

Acclimation Period

Upon procurement, the laboratory rats underwent a comprehensive acclimation process to familiarize them with their new environment. This period spanned three months, during which attention was given to facilitate their adjustment and minimize their stress. The extended duration of acclimation ensured that the rats had ample time to become fully accustomed to their surroundings, optimizing their readiness for subsequent research protocols. After the acclimation period of three months the specimens were ready to be used.

Frog Identification, Collection and Processing

Frogs were systematically collected and taxonomically classified to ensure the acquisition of appropriate specimens, with confirmation duly provided by Emerson Sy (Editor, Reptile and Amphibian Database – Philippines) and Andrei Bon A. Flores (PhD Biodiversity Cand., Department of Life Science, National Taiwan Normal University), identifying the frog specimen utilized in this research as *Hoplobatrachuschinensis*, commonly known as Chinese Edible Frog (*refer to Appendix A for reference*). Subsequently, the fresh dorsal skin was surgically removed from the frogs. The skin was then thoroughly cleansed using distilled water to eliminate extraneous substances and impurities (*See Appendix B*). Controlled drying of the skin was employed using a dehydrator to safeguard its integrity, and it underwent a precise pulverization process, ultimately yielding a finely powdered form well-suited for integration.

Confirmatory test

The study used Ninhydrin tests for the detection of amino acids and proteins present in *H. chinensis*frog skin, whereas biuret tests were used to detect the presence of peptide bonds.

Preparation and Treatment Application

Anesthesiology

A commercially available local anesthetic cream, J-PRO Lidocaine Numbing Cream, containing 25 mg of lidocaine and 25 mg of prilocaine, was applied topically on shaved, intact skin before puncture procedures. It took 30-60 minutes or more of skin contact to reach full effectiveness. This cream served as a substitute for injectable local anesthetics during surgical procedures, as it can penetrate the skin, enhance the pain relief provided by general anesthetics, and reduce the necessity for frequent opioid and/or non-steroidal anti-inflammatory drugs (NSAID) redosing.

Wound Preparation

The dorsal skin on the thoracolumbar region of all the albino rats was carefully shaved using scissors and a blade. The skin was disinfected with 70% ethyl alcohol and treated with a topical anesthetic prior to puncture. A sterilized blade, ruler, and pen were utilized to accurately measure the wound site, followed by precise incisions using a blade to create a square with dimensions of 7.6mm by 7.6mm. The surgical procedure was performed under aseptic conditions. To prevent any interference by other rats, the animals were housed individually.

Treatment Application

The randomly divided four groups of rats were subjected to treatment with various dosages of frog skin ointment. The control group received only liquid paraffin wax.

In Treatment 1, animals were treated with a combination of 0.002 grams of powdered frog skin (PFS) and 0.5 ml of liquid paraffin wax. In Treatment 2, the rats were treated with 0.006 grams of PFS and 0.5 ml of liquid paraffin wax. In Treatment 3, the rats were treated with 0.10 grams of PFS and 0.5 ml of paraffin wax.

Table 1. Treatment Dosages and Composition

Dosage	**Treatment 1 (PSFA)**	**Treatment 2 (PSFB)**	**Treatment 3 (PSFC)**	**Treatment 4 (CONTROL)**
Powdered Frog Skin (grams)	0.002 grams	0.006 grams	0.0100 grams	-
Liquid Paraffin Wax (ml)	0.5 ml	0.5 ml	0.5 ml	0.5 ml

Wound Contraction and Epithelialization Measurement

The study employed a precision instrument, such as a digital caliper, for the accurate measurement of wound contraction and epithelialization. The measurement process of wound contraction for both the control and treatment groups were involved continuous assessments at 24-hour intervals until complete wound closure was attained. The rate of contraction was calculated using the given formula below:

$$\frac{Total\ Wound\ Contraction\ per\ Day}{No.of\ Days}$$

While Contraction rate per day was calculated using the formula:

Initial wound area- Present Wound Area

The Measurement of the epithelialization period in excision wound model was monitored through the number of days until wound apposition.

Euthanasia

Upon the completion of data collection, the researchers proceeded with the euthanasia of the animal models This was accomplished through the administration of sodium pentobarbital (PB) via intraperitoneal injection, this method was chosen for its reliability, rapid onset of action, and effectiveness in including a painless death, adhering the ethical standards for humane euthanasia in animal research.

Statistical Treatment

Comparison between the treatment groups and the control group in terms of three parameters of wound healing — wound contraction rate, wound epithelialization, and wound healing — was performed through one-way Analysis of Variance (ANOVA). To identify the significant differences among the groups, Tukey Honest Significant Difference test was employed. The homogeneity of variance was checked using Levene's test, while Grubb's test was performed to identify significant outliers on the data. Data analysis was conducted using R studio and Microsoft Excel.

RESULTS AND DISCUSSION

Detection of Bioactive Compounds

Table 2 shows the result of the presence of bioactive compounds. Bioactive compounds are naturally occurring molecules in plants and other organisms that can have beneficial effects on human health. Identifying these compounds is crucial for understanding the potential health benefits of the samples being studied. The following results will show the relative abundance of Peptides, Proteins and Amino Acids in Chinese Edible Frog Skin.

Table 2. Detection of Proteins, Peptides and Amino Acids

Test	Procedure	Observations	Result
Biuret test	1 mL Powdered Frog Skin Extract Solution + 1-2 mL *Biuret reagent*	Purple coloration	Positive
Ninhydrin test	1 ml Powdered Frog Skin Extracted Solution + 1-2 mL *Ninhydrin reagent*	Purple coloration	Positive

As shown in the Table 2, the powdered frog skin solution exhibited Ruhemann's purple coloration for Ninhydrin test yielding a positive result. Additionally, the test sample tested using biuret test yielded a violet coloration indicating the presence of two or more peptide bonds. Furthermore, one of the test samples yielded a yellow color indicating the presence of phenyalalanine. The overall result indicates that bioactive compounds such as peptides and amino acids are present on the skin of Chinese Edible Frog (*Hoplobatrachuschinensis*). The presence of amino acids and peptides in the frog skin suggest that frog skin can improve wound healing. As indicated by Hung and Huang, various amino acids have been reported to have specific benefits on traumatic wound healing and burns as they improve collagen synthesis at the wound site. Moreover, Lee and Smith state that cyclic peptides promote faster healing of skin wounds as they bind to damage collagen, which is typically impaired during wound formation.

Wound Contraction Rate

Table 3 presents the data on wound contraction rate across three different treatments and Control.

Table 3. Average Wound Contraction Rate per Day of Treatment and Control Group

Groups	Count	Sum	Average	Variance
PFS C	3	17.74	5.91333	0.11803
PFS B	3	14.08	4.69333	0.04823
PFS A	3	11.78	3.92667	0.01763
CONTROL	3	8.4	2.8	0.0873

* Powdered Frog Skin (PFS)

Table 3 shows that PFS A had an average contraction rate of 3.92667 mm^2 per day, PFS B had 4.693332 mm^2 per day, PFSC had 5.91233 mm^2 per day, and the control group had an average of 2.8 mm^2 per day. This suggest that PFS C, which received the highest quantity of Powdered Frog Skin (0.1g) exhibited the highest average wound contraction rate compared to the other treatments, indicating potentially faster wound contraction rate. In contrast, the Control group displayed a slower average contraction rate of 2.8 mm^2 per day. This suggests that the application of frog skin may potentially accelerate wound contraction as indicated by the higher average contraction rate observed in the treatment group compared to the control group. This finding is aligned with the results of the study of Krishnan et al. (2020), which demonstrated significant increase in wound contraction rates in the groups treated with frog skin extract compared to the control group. The extracts promoted faster wound closure and enhanced tissue regeneration.

Wound Epithelialization

The table presents the average number of days required for wound epithelialization in each treatment group compared to the control group. Epithelialization refers to the process by which skin cells migrate and cover the wound surface, promoting healing.

Table 4. Average Days to Achieve Complete Wound Epithelialization Across Treatment Groups and Control

Groups	Sum	Average	Variance	Ranking
PFS C	17.74	9.67	0.33	1
PFS B	14.08	12.67	0.33	2
PFS A	11.78	14.67	0.33	3
CONTROL	8.4	22	1	4

* Powdered Frog Skin (PFS)

Table 4 presents data on wound epithelialization for four different treatments. The average number of days for wound epithelialization is approximately 9.67 days in PFS C, 12.67 days in PFS B, 14.67 days in PFS A and 22 days in the control group. This indicates that among the treatments containing different amounts of powdered frog skin. PFS C exhibited the fastest wound epithelialization, with an average of 9.67 days. This suggests that higher concentrations of PFS may contribute to faster wound epithelialization.

This explains that animals receiving frog skin-derived products achieved faster and more complete epithelialization, supporting the notion that higher concentrations of frog skin extract accelerate wound epithelialization (Li et al., 2019).

Wound Recovery

Table 5 presents the average wound recovery time (in days) for each treatment group and the control group. Wound recovery time refers to the duration it takes for a wound to heal completely. By analyzing these results, we can assess the effectiveness of the different treatments in accelerating wound recovery.

Table 5. Average Wound Recovery Time across Treatments and Control Group (Days)

Groups	Count	Sum	Average Days	Variance
PFS C	3	35	11.67	0.333333333
PFS B	3	43	14.33	0.333333333
PFS A	3	50	16.67	0.333333333
CONTROL	3	72	24	1

* Powdered Frog Skin (PFS)

Table 5 reflects the data on wound healing in four different treatments. PFS A has an average wound healing time of 16.67 days, 14.33 days in PFS B, 11.67 days in PFS A, and the CONTROL group has the highest average of 24 days. This implies that PFSC exhibited the shortest average healing time of 11.67 days, suggesting a potentially faster wound healing rate compared to the other treatments. The current research findings aligned with prior studies by Sai et al. (1995) and Venkat Raghavan et al. (2010), which also reported positive effects of frog skin treatment on wound healing. Early research showed a considerable rise in hydroxyproline, hexosamine, and uronic acid, followed by progressive decline, which resulting in a faster wound contraction and complete healing in 12 days compared to the control group, which took 20 days.

Comparison of Treatment and Control Group across Wound Healing Parameters (Wound Contraction, Wound Epithelialization and Wound Contraction)

This section comprehensively evaluated the effectiveness of various treatments in accelerating wound healing. The researchers analyzed three key parameters: wound contraction, epithelialization, and recovery time. By comparing the average values of these parameters across treatment groups and a control group, the study aimed to identify treatments that significantly enhance wound healing compared to the control. Additionally, it included multiple comparisons between the treatment groups to determine specific formulations that exhibited statistically superior healing properties. Table 6 shows the comparison on wound contraction rate.

Comparisons on Wound Contraction Rate

Table 6 presents the results of a one-way analysis of variance (ANOVA) comparing the effects of different treatment groups and control group on one of the three wound healing parameters: the wound contraction rate. This analysis statistically evaluated whether the observed differences in average wound contraction among the treatment groups and the control group were likely due to chance or if they indicate a significant impact on wound healing.

Table 6. Comparison of Wound of Contraction Rate Between Treatments and Control

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	15.43	3	5.14	75.85	0.000	4.07
Within Groups	0.54	8	0.068			
Total	15.97	11				

Table 6 presents the comparison of the results of ANOVA for assessing the wound contraction measurements across treatments with varying amounts of powdered frog skin. The table shows that the p-value of 0.000, significantly lower than the predetermined significance level of 0.05. This suggests that there was a statistically significant difference among the Treatments and Control in terms of wound contraction rate (*refer table 6.1 for details).*

Multiple Comparisons on Wound Contraction Rate

Table 6.1presents the results of Tukey's Honestly Significant Difference (HSD) to facilitate pairwise comparisons between all groups, identifying the significant differences in wound contraction rates not only between treatment groups and the control but also between specific treatment combinations.

Table 6.1. Multiple Comparison of Treatments and Control on Wound Contraction Rate

Pair	Difference	SE	Q	Critical Mean	p-value
PFS C vs PFS B	1.22	0.1503	8.1153	0.6808	0.002
PFS C vs. PFS A	1.9867	0.1503	13.2151	0.6808	0.000
PFS C vs. CONTROL	3.1133	0.1503	20.7096	0.6808	0.000
PFS B vs. PFS A	0.7667	0.1503	5.0998	0.6808	0.028
PFS B vs. CONTROL	1.8933	0.1503	12.5943	0.6808	0.000
PFS A vs. CONTROL	1.1267	0.1503	7.4945	0.6808	0.003

* Powdered Frog Skin (PFS)

Table 6.1 presents the pair wise comparisons of wound contraction between different treatments. The difference in average wound contraction between PFS C and PFS B is 1.22 mm^2, the difference in average healing days between PFS C and PFS A is 1.9867 mm^2. The difference in average healing days between PFS C and the control group is 3.1133 mm^2. The difference in average healing days between PFS B and PFS A is 0.7667 mm^2. PFS B and the control group is 1.8933 mm^2, and PFS A and the control group is 1.1267 days. Since p-value for all compared treatments is lower than the p-value of 0.05 the treatments were highly statistically significant, implying a significant difference between the treatments.

Among the treatments examined, Treatment PFS C containing the highest amount of powdered frog skin consistently exhibits significantly lower average healing times compared to both PFS B and PFS A, as well as the control group. These findings underscore the importance of treatment selection in optimizing wound contraction, with PFSC showing particularly promising results in this area. The findings are aligned with previous research by Sai et al. (1995) and Venkat Raghavan et al. (2010), which also reported positive effects of frog skin treatment on wound healing. These studies indicated a reduction in wound area after six days of treatment compared to control groups, with significant differences in the percentage of wound closure observed on days 4 and 6 post-surgery. This correlation between the current research findings and the findings of previous studies reinforces the effectiveness of frog skin as a biological dressing for promoting wound contraction and accelerating the healing process.

Comparisons on Wound Epithelialization

Table 7presents the results of a one-way analysis of variance (ANOVA) specifically examining wound epithelialization. This analysis statistically assesses whether the observed differences in average epithelialization time across treatment groups and the control group indicates a significant effect of the treatments on promoting wound healing.

Table 7. Comparison of Average Wound Epithelialization between Treatments and Control

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	248.25	3	5.444444	165.5	0.00	5.14
Within Groups	4	8	0.111111			
Total	252.25	11				

Table 7 displays the result of ANOVA for comparing wound epithelialization between the treated groups and the control. This analysis is supported by data showing that the critical F-value of 5.14 is less than the F-value of 165.5 and the P-value of 0.00 is less than the significance level of 0.05. This suggests a significant difference in the days required to achieve wound epithelialization between the treated groups and the control, leading to the rejection of the null hypothesis. Similar findings were reported in an experimental study by Zhang et al., which investigated the effect of frog skin extract (FSE) on wound healing and epithelialization in animal models. Animals with standardized wounds were treated with varying concentrations of PFS and wound healing outcomes, including epithelialization time, were assessed. Results showed a significant difference in epithelialization times among the treatment groups, with animals treated with higher concentrations of FSE exhibiting faster epithelialization compared to lower concentrations and control groups.

Multiple Comparisons on Wound Epithelialization Time

Table 7.1presents the results of Tukey's HSD between all groups, identifying significant differences in average days to achieve wound epithelialization. This analysis not only compares wound epithelialization between treatment groups and the control but also highlights difference between the treatment groups and control group.

Table 7.1. Multiple Comparison of Treatments and Control Group on Wound Epithelialization

Pair	Difference	SE	Q	Critical Mean	p-value
PFS C vs. PFS B	3	0.4082	7.35	1.85	0.003
PFS C vs. PFS A	5	0.4082	12.25	1.85	0.000
PFS C vs. CONTROL	12.33	0.4082	30.21	1.85	0.000
PFS B vs. PFS A	2	0.4082	4.90	1.85	0.035
PFS B vs. CONTROL	9.33	0.4082	22.86	1.85	0.000
PFS A vs. CONTROL	7.33	0.4082	17.96	1.85	0.000

* Powdered Frog Skin (PFS)

Table 7.1 shows the pairwise comparisons of wound epithelialization (days) between different treatments and the control. The difference in average days to achieve wound epithelialization between PFSC and PFSB is 3, and p-value of 0.003. Similarly, the difference in average wound epithelialization time between PFSC and PFSA is 5 days with a p-value of 0.000. Comparatively, the difference in average wound epithelialization time between PFSC and the control group is 12.33 days (p-value = 0.000), between PFSA and the control group is 7.33 days (p-value of 0.000), between PFSB and Control is 9.33 days (p-value of 0.000). Since the p-value for all compared treatments is lower than the 0.05, the treatments are highly statistically significant and among the treatments examined, Treatment PFSC consistently emerges as the most effective in promoting wound healing, as it exhibits significantly lower average healing times compared to both PFSB and PFSA, as well as the control group.

These findings highlight the potential of peptides to regulate various skin mechanisms by modulating inflammation, epithelialization, and the aging process. According to Jiabirg An et al., (2023), peptides have the potential to influence numerous physiological processes and biochemical signaling pathway.

Comparisons on Wound Recovery Time (Days)

This section examines the impact of different treatments including various concentrations of Powdered Frog Skin (PFS) on wound healing speed presenting a comparison of wound recovery time (measured in days) across treatment and control groups. The aim is to identify if any specific treatment formulations significantly influence the rate of wound closure compared to the control group.

Table 8 explores the influence of different PFS concentrations in the treatments on wound recovery time. It compares wound healing outcomes in groups receiving treatments with varying amounts of PFS to a control group treated solely with liquid paraffin wax.

Table 8. Comparison of Average Wound Recovery Time between Treatments and Control

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	252.67	3	84.22	168.44	0.000	4.07
Within Groups	4	8	0.5			
Total	256.67	11				

The statistical analysis revealed a p value of 0.000 which is lower than the significance level of 0.05 indicating a significant difference among the treatments. This demonstrates statistical significance and leads to rejection of the null hypothesis, which assumes no difference between treatments, is rejected. To further discern the subtle differences in means of the treatments, a post hoc analysis was conducted (*refer to Table 8.1 for details).*

Multiple Comparison on Wound Recovery Time

Table 8.1presents the results of Tukey's HSD analysis between all groups, identifying significant differences in average wound recovery time. This analysis not only compares wound recovery time between treatment groups and the control but also highlights the difference between the treatment groups and control group.

Table 8.1. Multiple Comparison of Across Treatments and Control Group on Wound Recovery Time

Treatment	Difference	SE	Q	Critical Mean	p.adj
PFS C vs PFS B	2.67	0.408	6.53	1.85	0.007
PFS C vs PFS A	5	0.408	12.25	1.85	0.000
PFS C vs CONTROL	12.33	0.408	30.21	1.85	0.000
PFS B vs PFS A	12.33	0.408	5.72	1.85	0.016
PFS B vs CONTROL	9.67	0.408	23.68	1.85	0.000
PFS A vs CONTROL	7.33	0.408	17.96	1.85	0.000

* Powdered Frog Skin (PFS)

Table 8.1 shows the pair wise comparisons of wound recovery days between different treatments. The difference in average wound recovery days between Treatment PFSC and PFSB is 2.67 days, with a p-value of 0.007. Similarly, the difference in wound recovery days between PFSC and PFSA is significant with a p-value of 0.000. Additionally, the difference in average wound recovery days between PFSC and the control group is 12.33 days (p-value = 0.000), and between PFSA and the control group is 7.33 days (p-value = 0.000). Since the p-value for all compared treatments is lower than the p-value of 0.05, the treatments are highly statistically significant and among the treatments examined, Treatment PFSC consistently emerges as the most effective in promoting wound healing, as it exhibits significantly lower average healing times compared to both PFSB and PFSA, as well as the control group. These findings underscore the importance of treatment selection in optimizing wound healing outcomes, with PFSC showing particularly promising results in reducing healing durations.

This finding negates the meta-analysis conducted by Johnson et al. (2017), examined the collective findings of multiple studies on frog skin extract's effects on wound healing in animal models. The analysis revealed inconsistent results across studies, with some showing significant improvements in wound healing and others showing no effect. This challenges the conclusions drawn from the table above that shows consistent and significant improvements in wound healing with frog skin extract treatment.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Based on the findings of the study, the following conclusions were drawn:

Hoplobatrachuschinensis skin can contribute to wound healing properties due to the presence of bioactive compounds within its dermal layer.

Higher concentration of powdered frog skin can potentially accelerate the wound contraction rate.

Treatment with the highest quantity of powdered frog skin can contribute to quicker wound healing terms of epithelialization.

There is an observable effect of varying amounts of powdered frog skin on the average number of days of wound healing, higher quantity of powdered frog skin can accelerate wound healing.

The inclusion of powdered frog skin in wound treatment offers superior wound healing outcomes compared to using liquid paraffin wax alone, making it a promising natural alternative for wound management.

Recommendations

Based on the findings of the study, the following recommendations are proposed:

Implementation of a splinting technique around the wound area in rodent models to enhance experimental precision and consistency.

Investigation into the potential application of frog skin ointment in promoting wound healing across various animal models, expanding the scope of its efficacy assessment.

Exploration of the antimicrobial properties of frog skin through dedicated microbial studies to expound its mechanisms and potential clinical applications.

Integration of histological examination to provide deeper insights into the histopathological changes associated with frog skin application in wound healing, thereby enhancing understanding of its therapeutic mechanisms.

Inclusion of local drugs for wound management as control groups, enabling comparative analysis and validation of the efficacy of frog skin treatment against existing pharmaceutical interventions.

Authors Contribution:

RJBL - conceptualized the research study, performed the laboratory processes, wrote the manuscript

ICS – conceptualized the research study, performed the laboratory processes, wrote the manuscript and shouldered most of the expenses especially in the laboratory fees for the study

ALM - conceptualized the research study, performed the laboratory processes, wrote the manuscript

JPP – gave inputs and guidance from the conceptualization of the study to the experimentation, gave inputs on the refinement of the manuscript and edited the final manuscript.

LITERATURE CITED

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Appendix A: Certificate of Identification

Certification of Identification

19 January 2023

College of Teacher Education

NUEVA VIZCAYA STATE UNIVERSITY,

Don Mariano Perez, Bayombong,

Nueva Vizcaya, Philippines

Dear Sir/Madam,

This is to certify that IVIE C. SEANGOY from Nueva Vizcaya State University has presented a series of frog photos for identification. The species was morphologically identified as:

Family	**Scientific Name**	**English Name**
Dicroglossidae	*Hoplobatrachusrugulosus* (syn. *Hoplobartrachuschinensis*)	Chinese edible frog

This certificate is issued for academic purposes only. I hope this identification has been of assistance to your department.

All the Best,

ANDRIE BON A. FLORES, MSc.

Specialization: Biodiversity, Ecology and Herpetology

PhD Biodiversity Cand., Department of Life Science, National Taiwan Normal University. TIGP Biodiversity Program, Academia Sinica, Taiwan abaflores@usm.edu.ph

CERTIFICATE OF IDENTIFICATION

This is to certify that IVIE C. SEANGOY from Nueva Vizcaya State University has presented a series of frog photos for identification on 17 December 2023. The species was identified as:

Family	**Scientific Name**	**English Name**
Dicroglossidae	*Hoplobatrachuschinensis*	East Asian Bullfrog

This certificate is issued for academic purposes only.

Determined by:

Emerson Y. Sy

Editor, Reptile and Amphibian Database – Philippines