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Burn Wound Healing Potential of Paragis (*Eleusine indica*) Leaf Ethanolic Extract Ointment on Sprague Dawley (*Rattus norvegicus*) Rats

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ABTRACT

This study aimed to investigate the burn wound healing potential of the ethanolic extract and 10% ointment from Paragis (*Eleusine indica*) leaves. The research utilized the Complete Randomized Design in evaluating the percent wound contraction and epithelialization period (days) of burn wound inflicted on the test animals, Sprague Dawley rats. The 10% ointment prepared from the ethanolic extract of Paragis (*E. indica*) leaves was found to be non-cytotoxic in the acute dermal toxicity test at the different dosage levels: 2000 mg/kg (limit test), 1000 mg/kg, 500 mg/kg, and 200 mg/kg. The ointment (Treatment 1) produced with ethanolic leaf extract of Paragis, showed the highest percentage (contraction) for it reached 100% within 16 days which was the last day of monitoring, followed by Treatment 2 (Paragis leaf ethanolic extract), because it reached 93.32% wound contraction at the expected day of contraction (16th day). The positive control ranked third with 84.98% wound contraction at day 16, followed by negative control with 66.62% wound contraction.

Keywords: *Burn wound healing, Ethanolic extract, Sprague Dawley rats*

INTRODUCTION

Wound healing is a crucial mechanism for the maintenance of homeostasis. In this regard, the skin must sustain a "robust and effective repair mechanism to ensure restoration of tissues and regeneration of injured skin or tissues. Cutaneous wound healing emanates as the "skin repairs itself" after exposure to injury due to "surgery, trauma, and burns". Wound healing occurs as a systematic flow of processes from "inflammation, angiogenesis, proliferation and synthesis of collagen for final healing" (Hemamaliniet as cited in Ahmed et al., 2016). Paragis belongs to the Class Magnoliopsida, Family Poaceae, with the binomial name *Eleusine indica*. It is a weed that grows abundantly in warm areas, particularly "along river banks, roads, and settled areas throughout the Philippines". In the Philippines, the decoction of leaves serves as a diuretic (Surigao del Sur) and a treatment for kidney diseases and arthritis (Sablan, Benguet Province) whereas dried leaves and stems are burned as an insect repellant (Porac, Pampanga). The plant is considered to possess folkloric medicinal value in different countries. It is recognized as a "diuretic, antihelmintic, diaphoretic, and febrifuge". Paragis is also used against "diarrhea, dysentery, epilepsy, and intestinal occlusion" (Cameroon), "treatment of infertility in females," muscle sprains (Sri Lanka), hypertension (Myanmar), "treatment of prolapsed uterus" (Bangladesh), liver disorders, and snake-bite (India) (Bangladesh Ethnobotany Online Database; Biswas et al.; Mabeku; Obico & Ragragio; Upasani et al; Wai et al,, as cited in Stuart, 2013). These traditional uses of Paragis should be probed further.

Several studies provide evidence of the medicinal efficacy of Paragis. This plant is noted to possess "antiviral, antiplasmodial, antidiabetic, and antibacterial properties. It was also investigated due to its "anticancer potential" (Aye et al, Morah & Otuk as cited in Nas et al, 2020).

Evidently, Paragis is one plant that deserves further scientific inquiry. Thus, this study will add to the existing body of knowledge on the wound healing capacity of an ointment from the ethanolic leaf extract of Paragis.

Objectives of the Study

This study aims to assess the wound healing potential of Paragis (*Eleusine indica*) leaf ethanolic extract ointment. It intends to examine the physical characteristics of the extract, perform physico-pharmaceutical evaluations of the ointment, determine acute dermal toxicity, and assess wound healing by measuring wound contraction and epithelialization. Additionally, the research seeks to establish whether Paragis ethanolic extract and Paragis ointment exhibit significant differences in wound healing compared to a positive control group.

MATERIALS AND METHODS

Research Design

The experiment was performed using a Complete Randomized Design (CRD) with a total of twenty-four (24) Sprague Dawley rats, which were distributed in the following order: four (4) rats for the acute dermal toxicity test (200 mg/kg, 500 mg/kg, 1000 mg/kg, and 2000 mg/kg ointments) and twenty (20) with five (5) replicates each for the four (4) treatments (negative control, positive control (0.2% Nitrofurazone), 10% ointment, and ethanolic extract) for the burn wound model.

T1 — 10% ointment (from Paragis leaves ethanolic extract)
T2 — Ethanolic extract of the Paragis leaves
T3 — positive control (0.2% Nitrofurazone)
T4 — negative control (untreated).

Sampling Technique

A group of healthy Sprague Dawley rats, each weighing between 200 to 250 grams, were randomly chosen as the study subjects.

Locale of the Study

The Paragis leaves used in this study were gathered from the outskirts of Gosi Sur, situated in Tuguegarao City, Cagayan. The experimental procedures were meticulously carried out within the confines of a controlled laboratory environment.

Research Instruments

The experimental setup for this study adhered to ethical standards and guidelines, with an animal permit secured from the Institutional Animal Care and Use Committee (IACUC). To ensure accurate and precise results, various chemicals and specialized equipment were utilized for plant extraction and ointment preparation. Additionally, Lidocaine was employed for anesthesia purposes, while normal saline played a crucial role in wound cleaning. To measure wound areas with precision, a transparent ruler was employed as a valuable tool throughout the experiment.

Data Gathering Procedure

Plant Collection and Authentication

Fresh, mature leaves of Paragis, approximately five kilograms (5 kg) were randomly collected by the researchers from the border of Gosi Sur, Tuguegarao City, Cagayan. It was authenticated by the Department of Agriculture Region II. The duration of collection lasted for about three (3) days. The leaves were washed initially with tap water and then distilled water to ensure the removal of soil and other impurities that may affect the result of the experiment. The washed leaves were cut into smaller strips and allowed to air dry until a constant weight was observed.

Preparation of the Plant Extract and Ethanolic Extract

The method of ethanol extraction was adapted from the procedure presented by Guevara (2005). The air-dried leaves of Paragis were subjected to ethanolic extraction. About 300g of sample was soaked in 3000 ml (1:10 g/ml ratio) of 70% ethanol for 48 hours. The filtrate was collected after filtration of the residue using a Whatman No. 1 filter paper and then concentrated by Rotary evaporator. A flame test was conducted to ascertain the absence of ethanol in the extract of Paragis leaves. The extracts were then packed in a clean, dry, and tightly sealed bottle and refrigerated until further use in the bum wound model.

Physical Characteristics of Paragis Leaves Ethanolic Extract

Physical characteristics of Paragis extract such as its color and odor were evaluated by visual and olfactory observation. The color and odor of the Paragis leaf ethanolic extract was observed and noted. Production of 10% Ointment from the Paragis Leaves Ethanolic Extract (Fusion Method Adapted from Rao Kodati et al., 2011). In the preparation of 10% ointment, two grams (2g) Wool fat (Lanolin), two grams (2g) of Hard Paraffin, two grams (2g) of Cetostearyl alcohol, and thirty-four grams (34 g) White Soft Paraffin were used. Each ingredient was mixed, the mixture was heated gently with constant stirring to achieve the desired consistency, and then the preparation was cooled. Four milliliters (4 mL) of Ethanolic Extract of Paragis leaves was slowly added to the melted ingredients and then stirred thoroughly to obtain a homogeneous mixture. The ointment was then packed in a wide mouth container.

Physico-pharmaceutical Evaluation of the 10% Ointment

The pH was determined using a pH meter. External characteristics such as color, odor, smoothness, grittiness, and homogeneity were determined by visual inspection and tested for appearance with no lumps.

Experimental Protocol on the Acute Dermal Toxicity and Wound Healing Activity

Acclimatization of Animals

The researchers secured an Animal Permit from Institutional Animal Care and Use Committee (IACUC). Healthy Sprague Dawley rats were randomly selected for this experiment. As noted by Demilew et al. (2018), experimental animals weighed 200 -250g. The animals were kept in individual cages where they had free access to diet and water for two (2) weeks for acclimatization to the laboratory conditions.

Grouping and Dosing of Animals

Grouping and dosing of the animal specimen were done as follows: four (4) treatment groups, each with five (5) rats were used for the burn wound model. Animals in Treatment I received the 10% ointment from the ethanolic extract of Paragis leaves. Those in Treatment II were treated with Paragis leaf ethanolic extract. Animals in Treatment III received 0.2% Nitrofurazone (Positive Control) while those in treatment 4 (Negative Control) were left untreated after the burn infliction. The remaining four (4) Sprague Dawley rats were utilized for the acute dermal toxicity test. All the conducted experiments conformed to the

international accepted guideline for laboratory animal use and care published by the National Academies Press (as cited in Demilew et al., 2018).

Acute Dermal Toxicity Test (Adapted from OECD November 22, 2017)

One rat was assigned per dose. The test substance was applied to the skin (not less than 10 percent of the body surface area) in graduated doses on each experimental animal. At least three dose levels were used, excluding the limit test, appropriately spaced to produce a dose-response curve. A limit test of at least 2000 mg/kg was made. The observation period was at least fourteen (14) days. During the first day, the animals were observed for five (5) hours and then the observations were made daily.

Burn Wound Model (Adapted from Subalakshmi, 2014)

Hairs from the predetermined area (refer to figure 1) for burn wound infliction at the back of the Sprague Dawley rats were removed with a previously sanitized razor. The skin lateral to the spinal groove was shaved, cleaned with cool distilled water, and then dried. The burn infliction done by Subalakshmi et al. (2014) was adopted in this study. The grouped rats were injected with Lidocaine (anesthesia) in the dorsal region of their body. A cylindrical metal rod of about twelve (12) mm in diameter, which was heated for 30 seconds with a blow torch, was pressed to the shaved portion of the rat for six (6) seconds. All wounds were cleaned with normal saline suggested by Khoo et al. (2010). The animals were then placed individually in their own cages after the infliction of the burn wounds and for recovery from the anesthesia.

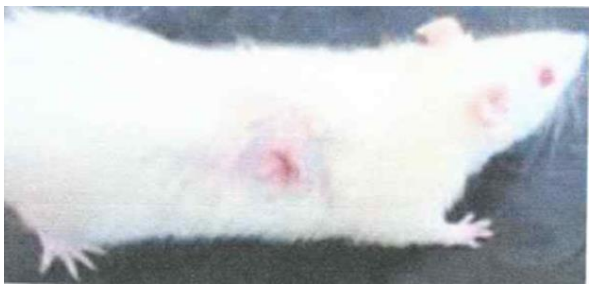


Figure 1. Site of burn wound infliction in

Burn Wound Management

The animals were monitored immediately postoperatively for spontaneous breathing efforts and movement. After surgery, each animal was housed in an individual cage in a room and fed with standard rat diet and water. All wounds were cleaned with normal saline and treatment was reapplied every three days.

Measurement of Wound Contraction (Adapted from Nagar et.al., 2016)

The burn wound healing potential was monitored by measuring the wound mean area and computing the percentage (%) of wound contraction among the four (4) treatments. The monitoring and assessment protocol suggested by Nagar et al. (2016) was used, except that the wound contraction evaluation was done on days 1, 4, 7, 10, 13, and 16 using a transparent ruler instead of a tracing paper. In case of infection occurrence, the monitoring was extended for two (2) more days (Subalakshmi et al. as cited in Demilew et al, 2018). Measurements of wound area were done on 1 mm2 scale. The surface area recorded on each rat was used to calculate the percent wound contraction, using the number of days (eighteen days) and taking the initial size of the wound as 100%, with the following formula:

% wound contraction
= (wound area on 1% day — wound area on day (n)/Wound area on 1* day x 100

Epithelialization Period

The falling of the scab, without traces of wound, served as the basis for the epithelialization period. This event was noted as the "end-point of complete epithelialization and the days required for this were taken as period of epithelialization" (Subalakshmi et al. as cited in Demilew et al., 2018).

Analysis of the Data/ Statistical treatment

The independent variable in the study was the type of treatment negative control (untreated), positive control (0.2% Nitrofurazone), ethanolic extract, and 10% ointment from the ethanolic extract of the Paragis leaves. The dependent variables, analyzed separately, were the percent of wound contraction and epithelialization period (days). The data on percent wound contraction on days 10, 13, and 16 were statistically analyzed. The data (percent wound contraction and epithelialization period) were analyzed using

One-way Analysis of Variance (ANOVA). At a 95% confidence level (significance level of a = 0.05), a p-value < 0.05 was used to reject the null hypothesis, which indicated a statistically significant difference in the efficacy of the test samples (10% ointment and ethanolic extract) against the positive control (0.2% Nitrofurazone). In case of rejection of the null hypothesis, further tests were done (Single Factor ANOVA Pairwise Comparison) to identify the treatments/groups that elicited the significant difference.

RESULTS AND DISCUSSION

Table 1. Physical Evaluation of Paragis leaf extract.

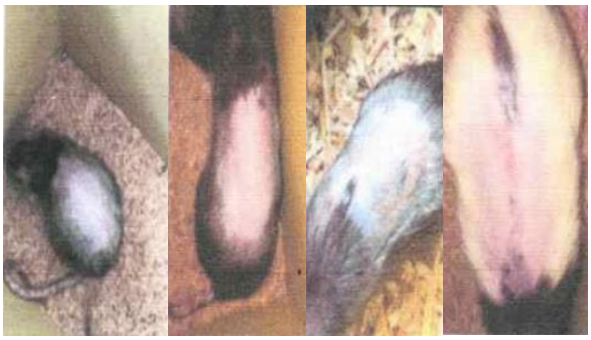
Physical Evaluation		
	Standard	Observation
Color	Dark green-brown	Yellowish-brown
Odor	Aromatic	Dried leaves tea

Table 1 shows the physical characteristics of Paragis leaf extract as to its color and odor as observed by the researchers and other respondents. The result showed that the color yellowish-brown and dried tea leaves odor was similar to the standard data of observation by V.P.S. Bhadauria, Varsha, Gupta et al., (2019).

Table 2. Physio-pharmaceutical evaluation of the ointment.

	Standard	**Observation**
Ph	5-7	Acidic (6)
Odor		Smells like wool fat
Smoothness	Smooth	Smooth
Grittiness	Free from grittiness	Non-gritty
Homogeneity and appearance with no lumps	Homogeneous	Homogeneous with no lumps

Table 2 shows the physico-pharmaceutical evaluation of the ointment fused with Paragis extract. The pH of the ointment was tested acidic with a pH of 6 and conformed to the standard pH as stated by Baravkar (2011). The odor was similar to wool fat odor due to its wool fat component. The ointment was homogenous without lumps, thus making it non-gritty, which is comparable to other prepared ointments in the study conducted by Baravkar (2011). According to Dr. Vijendra Nalamothu (2015), the importance of conducting the physico-pharmaceutical evaluation of ointment is that in the formulation components, a simple change in properties, such as pH, viscosity, the relative amounts of leaf extract, water, surfactants, stabilizers, droplet size, ionic nature, or the method of preparation, can often influence skin absorption and efficacy.



a b c d

Figure 2. Acute Dermal Toxicity of Ointment adopted from OECD 2017. *(a) 2000 mg/kg; (b) 1000 ng/kg; (c) 500 mg/kg and (d) 200 mg/kg*

The study also conducted dermal toxicity of the ointment with different levels of doses. The test served as a basis for establishing the dosage of Treatments, and it also provided information on dermal adsorption and the mode of toxic action of substance.

Figure 7 shows the result on dermal toxicity. It clearly shows that the ointment was not toxic for the test animals and did not show severe and enduring signs of distress and pain as it was the principle of the tests. A limit test was made which was 2000 mg/kg with following the three levels of doses (1000, 500, and 200), respectively, based on OECD (1987 updated November 22, 2017). As regards for the formula for getting the amount of dosage, the researchers converted the mg/kg to g/g depending on the weight of the rat.

In evaluating the wound healing process through percent wound contraction, the

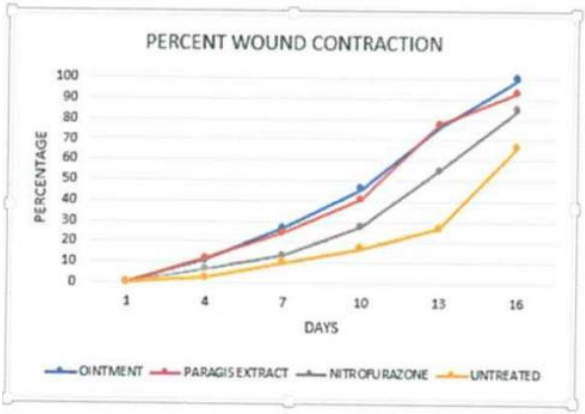


Figure 3. Evaluation of wound healing process through percent wound contraction

researchers used the protocol from Nagar, HK. et al, (2016). Figure 8 shows the evaluation of the wound healing process through percent wound contraction. As observed, the ointment (treatment 1) produced with ethanolic leaf extract of Paragis, showed the highest percentage (contraction) for it reached 100% within 16 days, which was the last day of monitoring, followed by Treatment 2 (Paragis leaf ethanolic extract), because it reached 93.32% wound contraction at the expected day of contraction (16th day). The positive control ranked third with 84.98% wound contraction on day 16, followed by negative control with 66.62% wound contraction.

The trend for percent wound contraction was: TI > T2> T3 > T4 (in which TI had the fastest rate of wound repair as seen in figure 8). For the falling off of scabs, the trend was: TI > T2 > T3 > T4 (in which TI or 10% ointment had the lowest or fastest period of epithelialization). The wound healing was evident in Appendix B in which replicates 2, 3, and 5 for T1 (10% ointment) and replicates 1, 4, and 5 for T2 (ethanolic extract) were completely cured on Day 13. At Day 16, all replicates for T1 (10% ointment); 1, 3, 4, and 5 for T2 (ethanolic extract); 1,2, and 5 for T3 (0.2% Nitrofurazone), and 3 for T4 (untreated) were totally cured.
The data was analyzed using one-way ANOVA. The efficacies were obtained by comparing the time of wound contraction using the four Treatments. The values were converted into percentages using the mean index, to show significant differences among all treatments. Treatment means that are significant, were compared using Least Significant Differences (LSD). Based on the analyzed data shown in table 3, Treatment 1 and Treatment 2 are not significantly different from Treatment 3. Treatments 3 and 4 also showed that they are significantly different at 0.05 level of significance.

Based on the results shown in table 3, treatments with the highest mean value have the greater bum wound healing potential. The treatment 1 (Ointment) ranked the highest with 100% wound contraction followed by Treatment II (Ethanolic extract), having 93.32%, Treatment Il (Positive Control) with 84.98%, and Treatment IV (Untreated) with 66.62%, respectively.

LSD results suggest that Treatments I, II and TII are not significantly different in terms of wound healing activity. Treatment III and Treatment IV are not significantly different.

Table 3. Mean of each treatment.

Treatment	Mean (%)	Rank
1 Ointment	100.00[a]	1
2 Extract	93.32[a]	2
3 Positive	84.98[ab]	3
4 Negative	66.62[b]	4
Total;	86.23	

**Any two means having a common letter(s) are not significantly different, otherwise they are significantly different at 5% level of significance.*

In evaluating the Period of Epithelization, the falling of scabs was determined by researchers as cited in Demilew et al, 2018. Treatment 1 (R2 and R3) showed the starting point was on day 9, followed by Treatment 1 (RS) and Treatment 2 (R1, Rd, and RS) on day 10. Then, Treatment 3 (R1, R2, and RS) on day 13. It was followed by Treatment 1 (R1 and R4), Treatment 2 (R3) and

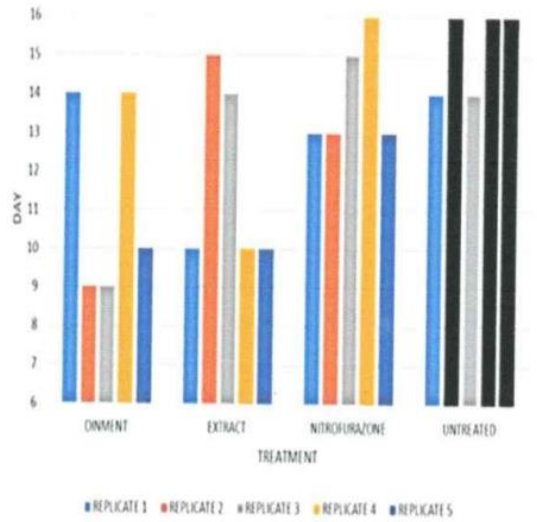


Figure 4. Data on Epithelization Period.

Treatment 4 (R1 and R3) on day 14. In addition, Treatment 2 (R2) and Treatment 3 (R3) on day 15. Moreover, Treatment 3 (R4) on day 16. Lastly, black bars represent Treatment 4 (R2, R4, and RS), which did not undergo epithelization during the experimental phase.

Normal epithelization occurs within 14 days as seen in studies conducted by Ozeion LLC, Wilmington, DE - (2013) and the results of the study conform to it. Early epithelization of some replicates in Paragis extract and Paragis ointment may be due to the presence of secondary metabolites such as flavonoids, alkaloids, and tannins as cited by Iberahim ct al. (2015).

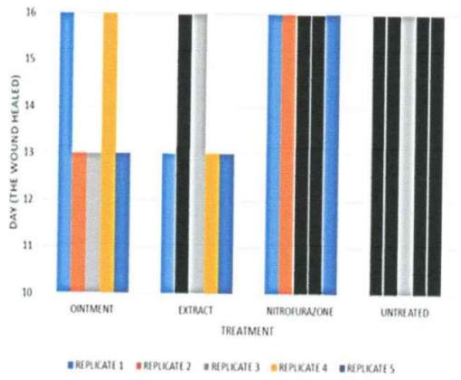


Figure 5. Data on Wound Healing

Based on the gathered results, the graph shows that Treatment 1 (R2, R3, and RS) and Treatment 2 (RI, R4, and RS) were fully healed on day 13. Treatment 1 (RI, and R4), Treatment 2 (R3), Treatment 3 (R1, R2 and RS) and Treatment 4 (R3) were fully healed on day 16. Moreover, black 'bars represent Treatment 2 (R2), Treatment 3 (R3 and R4) and Treatment 4 (R1, R2, R4 and RS) which did not heal during the experimental phase.

The normal fully healed wound occurs within 7-21 days as seen in studies conducted by - K. Kowalski et al. (2017) and the study results conform to it. The rapid healing of wounds seen in Paragis extract and Paragis ointment may be due to the presence of secondary metabolites like alkaloids, flavonoids, and tannins, as cited by Tberahim et al. (2015).

CONCLUSIONS

Based on the results, the researchers conclude that the Paragis leaf ethanolic extract and ointment have a burn wound healing property, with Treatment 1 being more effective than Treatment 2 due to the presence of an emulsifying agent that increases retention onto the skin of test animals. Test substance such as the ointment and ethanolic leaf extract was confirmed not toxic, for the test animals did not show severe and enduring signs of distress and pain.

RECOMMENDATIONS

The study on the burn wound healing potential of the ethanolic extract of Paragis (E. indica) leaves has yielded promising results, indicating the need for further exploration. To build upon these findings, several recommendations have been put forth. Firstly, it is advisable to extend the investigation to encompass other types of wounds, such as excision and incision wounds, to comprehensively assess the healing capabilities of Paragis Extract and its potential application in various clinical scenarios. Secondly, alternative methods of extraction should be explored to determine if different processes yield extracts with enhanced wound healing properties.

Moreover, additional physicochemical evaluations, including assessments of viscosity,

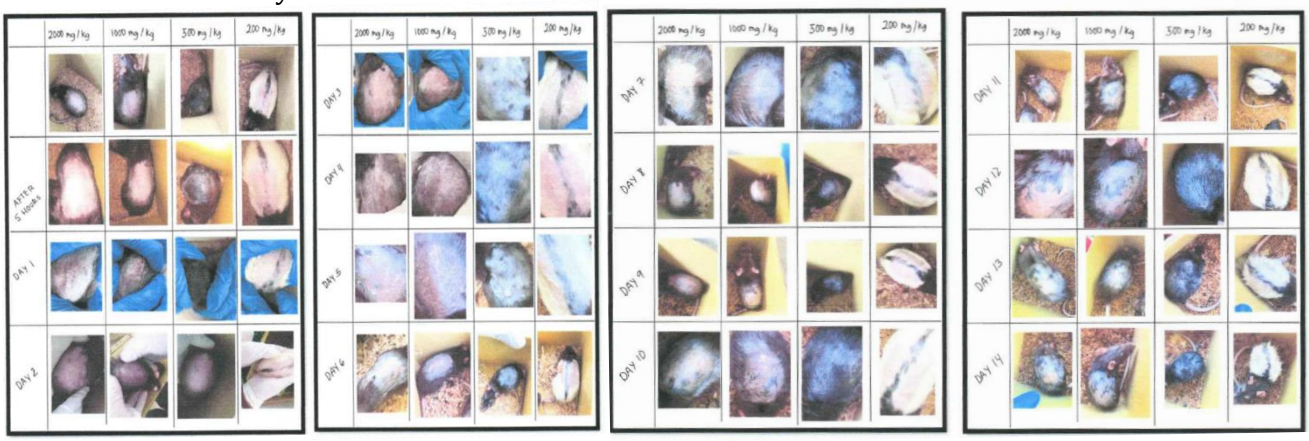
spreadability, and solubility of the ointment containing Paragis Extract, can provide valuable insights into its formulation and practical application. Furthermore, to gain a more comprehensive understanding of its therapeutic effects, it is recommended to examine various wound healing parameters, including wound coagulation, inflammation, granulation, and maturation.

Appendices

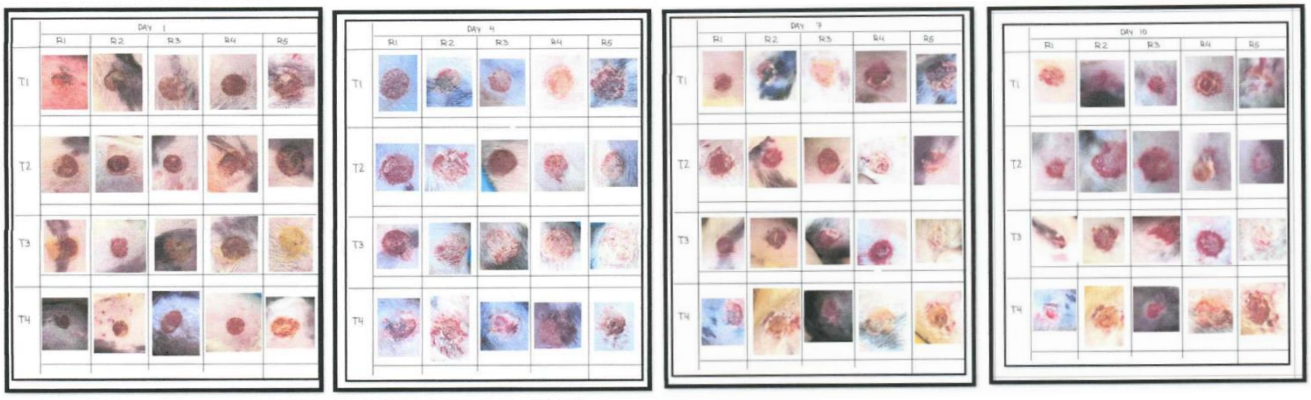
Appendix A. Percent Wound Contraction

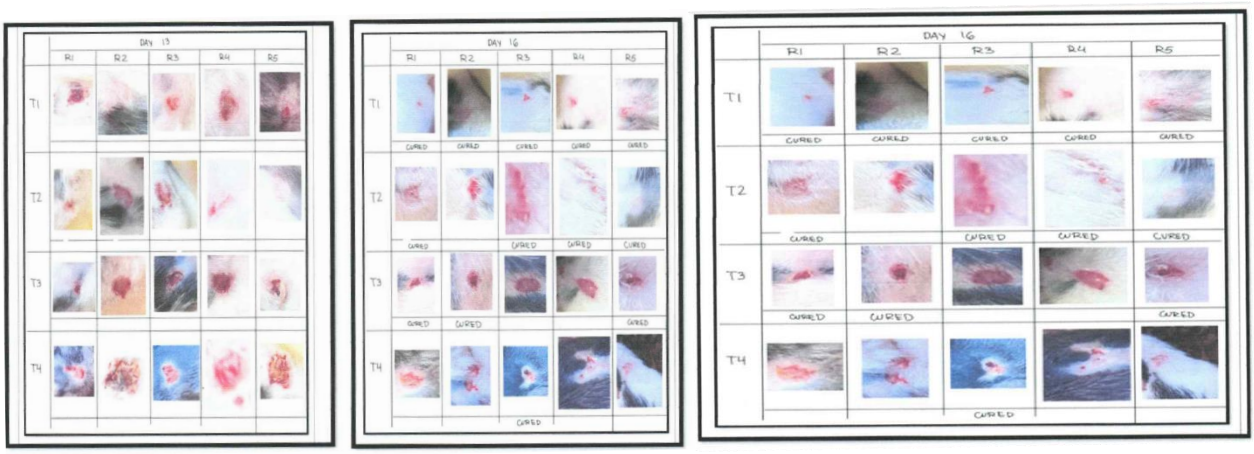
TREATMENTS/DAYS	1	4	7	10	13	16
OINTMENT	0	10.82	26.66	45.79	66.60	100
PARAGIS EXTRACT	0	11.66	24.99	40.81	77.48	93.32
NITROFURA ZONE	0	6.64	13.34	27.5	54.96	84.98
UNTREATED	0	2.49	9.99	16.68	26.66	66.62

Acute Dermal Toxicity



Burn Wound Healing





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