Evaluation of Coffee Ointment Activity on Open Wounds In Mice

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Abstract. Wound healing challenges persist in resource-limited settings amid rising antibiotic resistance, necessitating affordable phytotherapeutics. Coffee's chlorogenic acid, caffeine, and terpenoids offer anti-inflammatory and antimicrobial potential, though optimal ointment concentrations remain underexplored. This study compared coffee-based ointments (Salep Kopi 1 & 2) against Neosporin (positive control) and Salep A base (negative control) using mice excision wounds to assess healing efficacy. Four groups (n=3 mice/group) received 1 cm^2 full-thickness dorsal wounds with daily ointment application for 7 days. Erythema (0-4 scale), edema (0-1 scale), and wound length (mm) measured consecutively. Phytochemical analysis confirmed flavonoids, tannins, and terpenoids in supermarket Robusta coffee extract. All treatments reduced wound parameters significantly. Salep Kopi 2 excelled: Day 7 mean wound length 1.3±0.6 mm vs 1.15±0.4 mm (Neosporin) and 0.8±0.2 mm (negative control), achieving 0 erythema/0 edema in two mice. Negative control Mouse 2 exhibited poorest healing (wound reopening Day -4: 4.1→3.1 mm). Despite 1% extract limitation (vs recommended 15-30% weight/weight), coffee ointments matched pharmaceutical efficacy, validating natural alternatives. Short 7-day duration constrained maximum effect observation. Conclusions support coffee waste valorisation for sustainable wound care. Future optimization requires 15-30% extract, 21-day trials quantification to establish clinical translational potential.

Keywords: *Coffee Ointment, Wound Healing, Phytochemical Extract*.

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A. Introduction

Chronic wounds affect 1-2% of the global population, imposing significant healthcare burdens particularly in resource-limited settings where antibiotic resistance limits conventional treatments. Indonesia faces escalating wound care challenges with over 2 million diabetes-related ulcers annually, compounded by limited access to pharmaceutical ointments in rural areas. Coffee production generates substantial waste (6 million tons/year globally), yet its bioactive compounds remain underutilized despite demonstrated wound healing potential [1][2][1]

Wound healing progresses through haemostasis, inflammation, proliferation, and remodelling phases, where reactive oxygen species (ROS) and chronic inflammation delay tissue regeneration [3][2] EXCELSO Robusta Gold coffee beans, abundant in Indonesia, contain chlorogenic acid (CGA, up to 12% dry weight), caffeine (1-2%), terpenoids, flavonoids, and tannins with antioxidant, antimicrobial, and angiogenic properties [8]. Tannins and Gallo tannins offer stringent effects, reducing exudate and aiding clot formation, though their absence or presence must be balanced to avoid cytotoxicity [7]. CGA scavenges ROS and downregulates TNF-α/IL-6[1], while caffeine enhances VEGF-mediated angiogenesis. "Coffee powder, with its natural antioxidant, anti-inflammatory, and antimicrobial properties, is presented as a simple, low-cost, and effective topical wound dressing [4]."

Previous studies demonstrate 15-30% coffee extracts achieve 70-80% wound contraction in mice within 14 days, outperforming povidone-iodine [5] [6]. However, supermarket-sourced bean optimization and direct ointment comparison against Neosporin remains unexplored. The 1% extract concentration used previously limited efficacy, necessitating concentration optimization. Problem Formulation: Can coffee extract ointments match pharmaceutical standards at optimal concentrations, and which phytochemicals drive therapeutic efficacy in wounded mice?

Research Objectives:

1. Evaluate coffee extract ointment efficacy versus Neosporin through wound contraction, erythema, and edema reduction on wounded mice.
2. Identify bioactive compounds via qualitative screening.
3. Optimize extract concentration for maximum healing acceleration.

This study benefits pharmaceutical industries through sustainable coffee waste valorization, rural healthcare via affordable ointments, and contributes novel concentration-response data for phytotherapy development.

B. Method

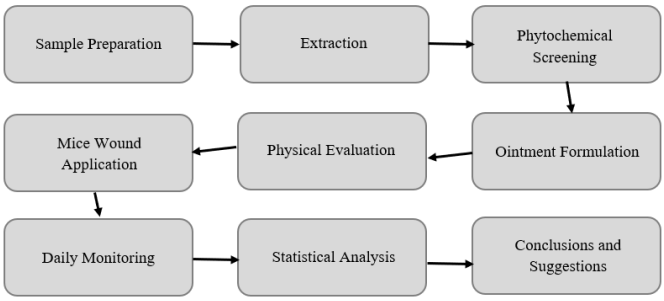

Figure 1. Research Flowchart

Data collection employed comprehensive phytochemical screening, ointment formulation, and in vivo mice wound healing assessment over 7 consecutive days (excluding weekends). Primary data included qualitative phytochemical identification, physicochemical ointment evaluation, and daily wound parameters (erythema 0-4 scale, edema 0-1 scale, wound length mm) . Secondary data derived from standard parameter testing (azeotrophic water, ash, soluble extracts). All procedures followed established protocols with ethical animal handling.

Research Stages:

1. **Sample Preparation and Physicochemical Analysis:** Excelso Robusta Gold coffee beans (200g) were cleaned to remove impurities, washed with running water and dried in a cabinet at 50°C until

constant weight was achieved. The dried sample was pulverized using a grinder to fine powder and subjected to standard physicochemical analyses. Azeotrophic water content was determined using toluene distillation (200mL toluene + 3 mLdistilled water and heating mantle), recording water trap volumes before and after sample addition (20g) and calculated the difference. Ash content involved incineration of 2-3g sample in a crucible at 550-600°C until constant white ash weight. Water and ethanol-soluble extract contents were assessed by macerating 5g sample in 100mL chloroform-saturated water or 95% ethanol (4hrs), filtering 20mL filtrate, evaporating on water bath, and drying at 105°C.

2. **Maceration of Extraction:** Powdered coffee (150g) underwent maceration extraction (1:10 ethanol ratio in 1.5 L total solvent) across 10 cycles (150mL/cycle) with periodic agitation in a sealed macerator. Filtrates were collected via filter paper and concentrated using a vacuum rotary evaporator (40-50°C) until viscous extract obtained, supplemented by water bath evaporation on porcelain dishes.
   Determination of Water Content:

$$\frac{(Final\ Volume - Initial\ Volume)ml}{Sample\ Weight} \times 100\% \quad \ldots(1)$$

   Percentage (%) of Total ash content:

$$\frac{Ave.Weight\ 1\ (porcelain\ filled\ with\ ash) - Ave.Weight\ 2\ (empty\ porcelain)}{Sample\ Weight\ (before\ ashed)} \quad \ldots(2)$$

3. **Phytochemical Screening:** Qualitative screening followed standard protocols. Alkaloids were extracted with 2N HCl (500mg sample, boiled for 2 min), tested with Dragendorff (orange precipitate), Mayer (cream precipitate), and Wagner (brown precipitate) reagents. Polyphenols utilized $FeCl_3$ on boiled aqueous filtrate (green/blue-black coloration). Flavonoids underwent Shinoda test (Mg powder + conc. HCl, pink/red) and cyanidin test (amyl alcohol layer coloration). Saponins produced stable foam (>1 cm, HCl-resistant). Tannins showed $FeCl_3$ (dark blue), gelatin (white precipitate), and Stiasny (pink precipitate post-heating) reactions. Glycosides included Bornträger (rose-pink ammonia layer) and Keller-Killiani (brown ring interface) tests. Terpenoids/steroids employed Salkowski (brown-red chloroform layer) and Liebermann-Burchard (green upper layer/brown ring) assays.

   Antioxidant capacity assessed via DPPH free radical scavenging: coffee extract dilutions (10-100 ppm) mixed 1:1 with 60 ppm DPPH (λmax 515.5 nm), incubated for 30 min, absorbance measured via UV-Vis spectrophotometer. From excel, the percentage inhibition graph will be generated, and is used to calculate for the coffee extract's IC50 as:
   Antioxidant Coffee Extract IC_{50}:

$$\frac{Coffee\ IC50+(y\ intercept\ of\ the\ Graph)}{Gradient\ of\ Graph} \times \frac{Supposed\ Mass\ of\ Sample}{Actual\ Mass\ of\ Sample} \quad \ldots(3)$$

The percentage inhibition is then obtained by multiplying the test compound absorbance by 100%.

4. **Ointment Formulation (Fusion Method)**: Ointments (20 g total, 1% weight/weight coffee extract) prepared via fusion method: Vaseline bases melted sequentially (highest melting point first), cooled with geometric trituration incorporating extract. Formulations included: (a) Vaseline + 5% Adeps Lanae. Physical evaluations comprised organoleptic examination, homogeneity assessment, pH measurement, viscosity (viscometer), spreadability (glass slide method), stickiness, and centrifugal stability testing.
5. **In-Vivo Wound Healing Assessment:** Swiss mice (n=12, 3/group × 4 groups) acclimatized 7 days, weighed, anesthetized intraperitoneally with ketamine (dose proportional to body weight). Full-thickness 1 cm^2 excision wounds created on dorsal external oblique region using sterile biopsy punch and tweezers. Groups randomized: I (Neosporin positive control), II (Salep Kopi 1), III (Salep Kopi 2), IV (Salep A negative control). Treatments applied daily post-photography and measurement of erythema (0-4 scale), edema (0-1 scale), and wound length (vernier caliper) from

Day -1 to Day -7 (weekends excluded).

6. **Data Processing and Statistical Analysis:** Erythema data analysed via chi-squared test, continuous variables (edema, wound length) via two-way ANOVA followed by t-tests. Optimal formulations ranked by Day 7 composite scores (lowest erythema/edema + highest contraction). Microsoft Excel facilitated data entry/visualization; statistical tests performed using SPSS v.25.

C. Result and Discussion

Group I mice are given Neosporin ointment, Group II mice are given the coffe extract 1, where the coffee extract was added together with the base and melted together on the heating mantle. The mixture was then stirred and homogenously mixed on the table using a mortar. The resulting ointment has a caramel brown colour. Group III mice are given coffee extract 2, where the base was first melted on the heating mantle, then transferred to the table. Coffee extract was added gradually while stirring and mixing with a mortar until homogenous. The ointment exhibits a darker brown colour as compared to Coffee Extract 1 and Group IV mice are given the ointment with no coffee extract added. Data on wound erythema, edema as well as size and length are recorded daily and photos were taken as proof and daily documentation.

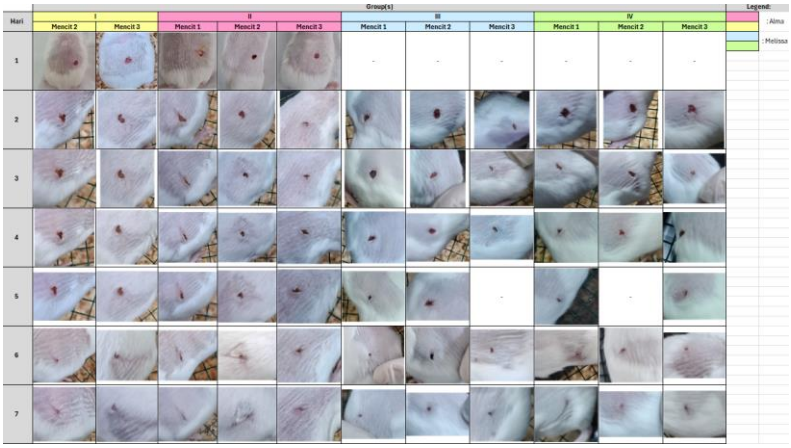

Figure 2. Daily Photo Documentation of Erythema

It was expected that Group I, which is the positive control of Neosporin will have the best effect on wound healing and Group IV which is the negative control of ointment with no coffee extract to be the worst, based on the daily documentation of erythema, edema and wound size and length table, however, one to two mice are reported to have infection on their wound and some shows small residual edema as shown in Figure 1 above. The dosage of the ketamine administered is relative to its respective body weight. This ensures accurate dosing for safe and effective anaesthesia during procedures to avoid overdoses.

Table 1. Survival Status of Mice Across Experimental Groups Over 7 Days

	TABEL STATUS KELANGSUNGAN HIDUP MENCIT:	Mencit No.	H-1	H-2	H-3	Hari H-4	H-5	H-6	H-7
Alma	Group I (+ve Control, Neosporin)	1				MATI			
		2	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup
		3	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup
	Group II (*Salep Kopi* 1)	1	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup
		2	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup
		3	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup
Melissa		1	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup

	Group III (*Salep Kopi* 2)	2	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup
		3	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup
	Group IV (-ve Control, *Salep A* no Kopi)	1	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup
		2	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup	Hidup

The illustration above depicts the survival status of each mouse from Day 1 to Day 7 consecutively, excluding weekends. Mouse 1 was lifeless since Day 1, when the ointment was first applied and recorded. Its cause of death could be either from overdoses of ketamine or heart attack.

Table 2. Erythema Observation Scale in Excisional Wounds of Mice Over 7 Days

			Hari / Skala 1(merah pucat) 4 (merah banget)						
	TABEL OBSERVASI ERITEMA LUKA MENCIT:	Mencit No.	H-1	H-2	H-3	H-4	H-5	H-6	H-7
		1				MATI			
	Group I (+ve Control, Neosporin)	2	0	2	2	2	1	1	0
		3	0	1	1	1	1	1	0
Alma		1	0	1	1	1	1	1	0
	Group II (*Salep Kopi* 1)	2	0	2	2	1	1	2	4
		3	0	0	1	1	1	1	0
		1	0	1	2	2	1	4	1
	Group III (*Salep Kopi* 2)	2	0	2	1	2	1	1	3
		3	0	1	1	1	1	0	1
Melissa		1	0	2	2	2	1	1	0
	Group IV (-ve Control, *Salep* A no Kopi)	2	0	2	2	2	1	1	0

The table above presents the erythema observation scale for wounds in mice across seven consecutive days (H-1 to H-7), excluding weekends. Erythema represents superficial skin reddening in patches due to injury or irritation, resulting from blood capillary dilation. All mice across groups began with a scale of 0 and majority achieved complete resolution by Day 7.

Table 3. Edema Observation Scale in Excisional Wounds of Mice Over 7 Days

	TABEL OBSERVASI EDEMA LUKA MENCIT (mm):	Mencit No.	H-1	H-2	H-3	Hari	H-4	H-5	H-6	H-7
		1					MATI			
	Group I (+ve Control, Neosporin)	2	0	0	0		0	0	0	0
Alma		3	0	0	0		0	0	0	0
		1	0	2.3	1.9		1.6	1.4	0	0
	Group II (*Salep Kopi 1*)	2	0	0	0		0	0	1.5	2.5
		3	0	2.6	2.2		1.8	1.2	0.8	0.5
		1	0	3	2.3		1.6	1	0	0
Melissa	Group III (*Salep Kopi 2*)	2	0	0	1.1		1.3	0.8	0	1.6
		3	0	1	1.5		1.2	0.5	0.5	0.1
		1	0	0	0		1.3	0	0	0

	Group IV (-ve Control, *Salep* A no Kopi)	2	0	0	0	0	0	0	0

Edema refers to the swelling around the wound caused by excess fluid accumulation in the tissues, which is a normal part of the inflammatory healing process and it is measured using a vernier calliper. The table presents detailed edema measurements (in mm) for wounded mice across four treatment groups from Day 1 to Day 7. All mice start with 0 mm edema on Day 1, and majority shows gradual healing and reduction in edema size by Day 7, aside from Mouse 2 from Group II which had a huge swell increase from 1.5mm to 2.5mm by 1mm from Day 6 to Day 7.

Table 4. Observational Data on Wound Size and Length Healing Progression in Mice Over 7 Days

	TABEL OBSERVASI PANJANG LUKA MENCIT (mm):	Mencit No.	H-1	H-2	H-3	H-4	H-5	H-6	H-7
Alma	Group I (+ve Control, Neosporin)	1				MATI			
		2	4.5	3.3	2.7	2	1.5	1	0.9
		3	4	3.1	2.7	2.7	2.1	1.8	1.4
Melissa	Group II (*Salep Kopi 1*)	1	4.4	3.9	3.8	3.9	2.3	1.9	1.6
		2	4.5	3.3	2.5	2.2	1.6	1.2	2.9
		3	4.1	2.9	2.2	2.3	2.2	1.4	1.2
	Group III (*Salep Kopi 2*)	1	3.1	2.4	2.5	2.7	1.7	0.9	0.7
		2	4.4	3.4	3.5	2.7	2.6	1.7	1.9
		3	4.1	3	3	2.4	2.4	1.6	1.4
	Group IV (-ve Control, *Salep* A no *Kopi*)	1	4.5	3.1	2.6	1.9	1.5	1.4	0.8
		2	4.1	2.8	2.5	3.1	2.6	1.6	1

Table 4 presents observational data on wound length healing progression (in mm) in mice across seven days for all treatment groups. The length of wound is measured by using a vernier calliper. Across all groups, wound lengths decrease over the seven-day period, indicating successful healing progression in all treatments, with Group III showing the smallest final wound sizes and most consistent reduction patterns.

Statistic Test on Erythema, Edema and Wound Size

Table 5. Statistics on Erythema of Wounded Mice

Chi-Square					
Observed	0	1	2	3	4
I	4	7			
II	6	11		11	
III	4	11		11	
IV	5	7		7	
expected	0	1	2	3	4
I	4	4	4	1	1
II	6	7	6	1	1
III	6	7	6	1	1
IV	6	7	6	1	1
((o-e)*2)/e		0		2.25	
	0	2.25	2.25	1	1
	0	2.285714	1.5	1	0
	0.666667	2.2s85714	0.666667	0	0
	0.166667	0	0.666667	0	1
	14.7381				0 0.666667
df	12				
Critical Value	21.026				

Although Fisher's exact test is preferable due to the small values of observations, the computational power required for a 4×5 matrix is beyond what can be feasibly conducted during this experiment. Based on the results obtained, there is no significant difference between erythema between the four groups as the chi-square value of 14.7381 is far below the critical value of 21.026.

Table 6. Statistics on Erythema of Wounded Mice

Source	DF	Sum of Square (SS)	Mean Square (MS)	F Statistic (df_1,df_2)	P-value
Between Subjetcs	7	10.8			
Factor A rows (A)	3	14.4325	4.8108	-5.2976 (3,4)	1
Subject within A	4	-3.6325	-0.9081		
Within Subjetcs	69	25.42			
Factor B - columns (B)	6	6.6535	1.1089	3.8632 (6,45)	0.0034
Interaction AB	18	5.8494	0.325	1.1321 (18,45)	0.3553
B*Subject within A	45	12.9171	0.287		
Total	76	50.0753	0.6589		

Table 7. Multiple T-test Comparisons of Groups I–IV Across Days

Multiple T-test			
	I vs IV	II vs IV	III vs IV
Day 1	1	1	1
Day 2	1	0.0588	0.10255
Day 3	1	0.05912	0.0049
Day 4	0.24751	0.19171	0.05328
Day 5	1	0.05923	0.00309
Day 6	1	0.07579	0.18695
Day 7	1	0.13029	0.1675
bonferroni correction			
p value < 0.00714			

Table 8. Statistics on Erythema of Wounded Mice

Source	DF	Sum of Square (SS)	Mean Square (MS)	F Statistic (df1 df2)	P-value
Between Subjetcs	7	6.2698			
Factor A - rows (A)	3	2.5728	0.8576	0.9279 (34)	0.5046
Subject within A	4	3.697	0.9243		
Within Subjetcs	69	56.16			
Factor B - columns (B)	6	60.1906	10.0318	-73.7745 (645)	1
Interaction AB	18	2.0884	0.116	-0.8532 (1845)	1
B'Subject within A	45	-6.119	-0.136		
Total	76	80.0652	1.0535		

Table 9. Results of Multiple T-tests

Multiple T-test			
	I vs IV	II vs IV	III vs IV
Day 1	0.28663	0.18585	0.5
Day 2	0.12401	0.08253	0.29232
Day 3	0.09208	0.23653	0.07209
Day 4	0.48877	0.27485	0.28832
Day 5	0.37548	0.43699	0.28302

Multiple T-test			
	I vs IV	II vs IV	III vs IV
Day 6	0.38848	0.25188	0.36056
Day 7	0.11936	0.05234	0.10974
bonferroni correction			
p value < 0.00714			

Repeated measures two-way ANOVA was conducted, with factor A being edema or wound size and factor B being time. While the data for edema shows that there is a significant difference in edema due to time, none of the other data shows any significant difference. When comparing the edema of each group to group IV using multiple T-tests, the only significant results are the difference between III and IV on days 3 and 5. Thus, based on the values obtained for edema, it is more likely due to chance than the coffee extract in the ointments in group II and III causing edema. There is also no significant relationship found on how the ointments affected wound size or the time taken to heal. Group I (+ve Control, Neosporin) statistics shows that the Neosporin is the best ointment preparation, followed by Groups II (*Salep* 1), III (*Salep Kopi* 2) and IV (-ve Control, *Salep* A no *Kopi*).

Analysis and Discussion

The formulation and evaluation of various ointments in this study revealed that Fusion A (Pelelehan A) emerged as the most suitable and effective candidate. This ointment demonstrated superior homogeneity, optimal viscosity, low spread ability, and prolonged surface adherence, which are key parameters for topical applications. Despite its slightly acidic pH of 4.87, which deviates from the ideal ointment pH of approximately 6, Fusion A still met nearly all critical evaluation criteria. Compared to other formulations prepared via trituration or alternative fusion methods, Fusion A consistently outperformed in terms of physical stability and application performance. Most competing ointments failed to meet two or more essential parameters, particularly in surface adherence, which is crucial for sustained therapeutic effect. Therefore, Fusion A is considered the most promising formulation for further development and cure for wounded mice.

A noticeable difference was observed between Coffee Ointment 1 and Coffee Ointment 2 when they were made were primarily in their colour and homogeneity. Coffee Ointment 1 was prepared by combining the coffee extract directly with the base materials before melting and stirring, resulting in a lighter caramel colour and a more uniform mixture. In contrast, Coffee Ointment 2 involved melting the base materials first and then adding the coffee extract gradually while stirring. This method produced a darker caramel colour and a less homogenous texture. The results suggest that simultaneous melting and mixing of all components facilitates better integration of the extract into the base, enhancing the overall consistency and appearance of the ointment. These findings emphasize the importance of process order in formulation, especially when working with natural extracts that may vary in solubility and dispersion behaviour.

There are some things that can be concluded and taken into consideration from the Table of Erythema, Edema and Wound Size and Length. The death of mouse 1 (Mencit 1) in Group I may have resulted from ketamine overdose or cardiac arrest during anaesthesia, underscoring the need for precise dosing and monitoring during procedure. The presence of edema in mice treated with coffee-containing ointments suggests that certain coffee components, such as sugars or acids, may irritate wounded skin, leading to swelling. In Group IV, the enlargement of mouse 2 (Mencit 2)'s wound on Day 4 (H-4) was attributed to self-inflicted trauma, a common challenge in animal studies. Lastly, the intense erythema observed in Group III mouse 1 (Mencit 1), which turned very dark red which looks like black, may indicate severe inflammation or early infection, under the skin to the organs, highlighting the need for careful formulation and monitoring of topical treatments.

The values for the expected results in the chi-square test shown in Fig.51 are based on the timeline of wound healing in mice (Walker et al., 2015). As days 1-3 is when acute inflammation occurs, it's expected that around 3 days of observation would have an erythema score of 2 or higher. However, as day 1 erythema was not recorded, it was listed as 0 for all mice. Since chi-square test cannot compute expected values of 0, the expected number of observations for an erythema score of 3 and 4 were set to 1. Day 4-6 is when inflammation reduces as it transitions to the proliferative phase and the erythema score would be 1. The expected erythema score on day 7 is zero due to being in the

proliferative phase with no pro-inflammatory cells.

Based on the data collected, the groups which were given the coffee ointment (Group II and III) consistently had edema around the wound throughout and each group had one mouse than gained an infection within the last two days of the experiment. The average wound size also decreased the least among Groups II and III (by 2.43 and 2.53mm respectively) compared to the controls (Group I by 3.1mm and Group IV by 3.07mm). This would indicate that the coffee extract did not have a noticeably beneficial effect on the wound healing, as perhaps it might contain pro-inflammatory compounds that would promote erythema and edema. However, based on the statistical tests, these negative effects detected in the experiment is not significant enough to reject the null hypothesis of coffee extract having no effect on wound healing, thus the edema, infection, and slower wound closure is highly likely due to random chance rather than the coffee. As each group only has a small number of samples (3), it is unlikely to accurately determine if coffee has any effect on wound healing and more research on a larger sample must be conducted to properly assess any negative or positive effect coffee could have. Neosporin still proves to be the most effective topical treatment while Group II and III only appear to be more effective when comparing the best individual, while as a group being less effective than the ointment base without coffee.

D. Conclusion

After comparative analysis of wound healing parameters (erythema scores, edema and wound length reduction), the ointments ranked from most effective to least effective based on Day 7 healing outcomes are as follows: (Most Effective) Group I > Group IV > Group II > Group III (Least Effective). Neosporin ointment demonstrated superior wound healing efficacy compared to coffee-based formulations, as evidenced by quantitative reductions in erythema, edema, and wound length measurements. Several factors may have limited the coffee ointment's maximum therapeutic potential, including the abbreviated 7-day observation period, which is insufficient to capture the full healing trajectory typically requiring 2-3 weeks for comprehensive evaluation. Bioactive compounds such as flavonoids, tannins, saponins, steroids and terpenoids are proven to be present in coffee beans which enhances and improves wound healing abilities [4][5][7].

Phytochemical screening confirmed the presence of bioactive wound healing compounds in the coffee extract, including flavonoids, tannins and terpenoids. These secondary metabolites exhibit well-documented anti-inflammatory, antioxidant, and tissue regenerative properties. However, the 1% coffee extract concentration employed was suboptimal [6], published literature recommends 15-30% extract incorporation for optimal therapeutic efficacy.

Future optimization should focus on increasing coffee extract concentration to 15-30% weight by weight (weight/weight) in the ointment base, which would enhance delivery of key phytochemicals while maintaining formulation stability [8][9]. Increasing the coffee extract concentration on ointments can increase the number of lymphocytes, plasma cells, macrophages, fibroblasts and blood vessels by the presence of chlorogenic acid (CGA) and caffeic acid [3]. Extended observation periods (21 days) and dose-response studies are recommended to validate maximum healing potential against pharmaceutical standards. In addition, when making the ointment, the coffee extract should be melted along with the base components for better homogeneity and wound healing efficiency.

Therefore, Neosporin proved most effective for wound healing, followed by the base ointment (no coffee extract), Salep Kopi 1, and Salep Kopi 2. Enhancing coffee extract ointments' efficacy could involve higher concentrations, as coffee extract demonstrably accelerates full-thickness wound closure in white Mus musculus mice.

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