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The Potential of Robusta Coffee Bean (Coffea Canephora) Extract in Vivo Healing of Incision Wounds in Sprague Dawley Rats

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Abstract

Cuts are a common type of wound and require proper treatment to accelerate healing and prevent infection. One plant that has potential for wound healing is *robusta coffee* (*Coffea canephora*). This study aims to evaluate the effectiveness of thick extract of *robusta coffee beans* on wound healing in Sprague Dawley white rats. The study used a laboratory experimental method with a Post-Test Only Control Group Design. A total of 25 rats were divided into five groups, namely negative control (no treatment), positive control (povidone iodine), and three treatment groups of *robusta coffee extract* with concentrations of 10%, 20%, and 30%. Treatment was given once a day for 14 days, and histopathological examination was performed on the 15th day. Macroscopic observation data used Nagaoka scoring, then all macroscopic and histopathological data were analyzed with SPSS using the Kruskal-Wallis and Mann-Whitney tests. The results showed that all concentrations of *robusta coffee bean extract* improved wound healing and tissue regeneration compared to negative and positive controls, with significant differences between groups ($p < 0.05$). Concentrations of 20% and 30% *robusta coffee bean extract* provided the most optimal effect in accelerating healing and tissue regeneration.

Keywords: robusta coffee bean extract, incision, Nagaoka scoring, macroscopic, histopathology

INTRODUCTION

Injuries are the most common type of trauma in Indonesia. According to the 2018 Basic Health Research (Riskesdas) data, abrasions accounted for 64.1% of all injuries, followed by dislocations at 32.8%, cuts or lacerations at 20.1%, fractures at 5.5%, and severed limbs at 0.5% (Riskesdas, 2019).

Cuts or scratches on the skin can be caused by a variety of factors, such as injury, surgical procedures, pressure, friction, or medical conditions like diabetes mellitus. The wound healing process can be slow or fast, depending on various factors. Open wounds that are not treated properly are at risk of infection. Understanding the factors that can speed up wound healing is important to prevent complications and minimize scarring so that skin conditions can return to normal. The wound healing process can be slow or fast, depending on various factors (Asyifa et al., 2023).

During wound care in the medical field, antiseptics such as povidone-iodine 10% are commonly used because they have antimicrobial and anti-inflammatory effects. However, in some individuals, povidone-iodine can cause side effects including fibroblast inhibition and

skin irritation. As a result, disruption of fibroblast activity can slow down the formation of new tissue and delay optimal wound closure (Hardiani et al., 2023). Fibroblasts are the most common type of cell found in connective tissue in the dermis layer of the skin. They are essential for the formation of collagen, which forms new tissue during the wound healing process. This collagen functions to reunite damaged skin tissue, strengthen skin structure, and accelerate the gradual wound closure process (Giri et al., 2021).

Coffee is a plant that has the potential to be used in wound healing therapy because it contains various bioactive compounds such as chlorogenic acid, caffeine, quinolic acid, trigonelin, tannins, and polyphenols. These compounds have anti-inflammatory, antioxidant, and antibacterial activities that play roles in reducing inflammation, preventing infection, and accelerating tissue regeneration (Fatharoni et al., 2023). Among the two main types of coffee widely cultivated in Indonesia, namely *Arabica coffee (Coffea arabica)* and *robusta coffee (Coffea canephora)*, *robusta coffee* is known to have a higher caffeine and chlorogenic acid content. This content is thought to provide a stronger bioactive effect in accelerating the wound healing process, so *robusta coffee beans* were chosen as the main ingredient in this study (Hasbullah et al., 2021).

According to Alphama's research, the fastest wound healing time was obtained in the group given *robusta coffee* powder with an average of 6.56 days, compared to the povidone-iodine group with 10.56 days and the control group with 11.67 days. The administration of *robusta coffee* powder increases the number of lymphocytes, plasma cells, macrophages, fibroblasts, and blood vessels that play roles in tissue regeneration. This effect is thought to be influenced by the content of chlorogenic acid (CGA) and caffeic acid which accelerate the healing process. Caffeine is an alkaloid compound of the xanthine group, acting as an antibacterial by inhibiting the synthesis of bacterial cell walls, preventing infection in wounds (Alphama et al., 2021).

Previous studies have explored the wound-healing potential of coffee but revealed varying results due to differences in extraction methods and dosage concentrations. Rahmawati et al. (2021) demonstrated that *robusta coffee* extract significantly accelerates fibroblast proliferation and collagen synthesis in incision wounds of white rats, attributing this effect to the high chlorogenic acid and caffeine content. However, the study did not compare its efficacy against standard antiseptics such as povidone-iodine, limiting its clinical applicability. In contrast, Sari and Wulandari (2022) found that while *Arabica coffee* extract exhibited antioxidant activity, its wound-healing effect was not statistically significant compared to commercial antiseptics, suggesting that coffee's bioactive compounds alone may not be sufficient without optimized concentration and formulation. Both studies highlight coffee's therapeutic promise but leave a gap in understanding the in vivo effectiveness of *robusta coffee* extract compared to conventional treatments.

Based on these findings, this study was conducted to determine the potential of *robusta coffee bean extract (Coffea canephora)* in healing slicing wounds in vivo in white rats of the Sprague Dawley strain. Its benefit lies in offering an affordable, locally available, and biocompatible alternative to chemical antiseptics, potentially contributing to the development of herbal-based therapeutics in Indonesia's health and pharmaceutical sectors.

RESEARCH METHOD

This study is an experimental study with a post-test-only control group design that aims to analyze the effect of robusta coffee bean extract on the healing of cut wounds and the formation of epidermal tissue, fibroblasts, and collagen in white mice of the Sprague Dawley strain. This research was carried out for 4 months in several locations, namely Gunung Wangun Dua Coffee Plantation, Karang Tengah, Babakan Madang District, Bogor Regency, West Java; School of Veterinary and Biomedical Medicine, Bogor Agricultural University; iRATco Laboratory, Bogor; Herbarium Depokensis (UIDEP), Department of Biology, Faculty of Mathematics and Natural Sciences, University of Indonesia; and Natural Materials Technology Laboratory and Pharmacology Laboratory, Faculty of Pharmacy, Pancasila University.

Tools and Materials

The tools used in this study include vacuum drying, analytical scales, maserators (IKA), filter paper, syringes, scalpel number 11, scissors, rat cages, rat scales, animal hair cleaning tools, measuring cups, beaker glass, drip pippettes, maceration jars, test tubes, test tube racks, Karl Fischer, desiccant, sieve no. 40 and pH meter.

The ingredients used include robusta coffee bean simplicia (Coffea canephora), Povidone iodine 10%, Ketamine HCl, ethanol 70%, swab alcohol, aquadest, BR-2 standard feed, NaCl solution, sterile gauze, and 10% Neutral Buffered Formalin (NBF) solution, reagents for phytochemical screening tests, namely Dragendorf reagents, Mg powder, concentrated HCl, FeCl 3 and 10% NaCl.

Research Procedure

1. Plant Determination: Determination of coffee plants was carried out at the Depokensis Herbarium (UIDEP), Department of Biology, Faculty of Mathematics and Natural Sciences, University of Indonesia.
2. Collection of Ingredients and Manufacture of Simplisia Powder: The robusta coffee beans (Coffea canephora) used in this study were obtained from the Gunung Wangun Dua Coffee Plantation, Karang Tengah, Babakan Madang District, Bogor, West Java, and have been roasted at a medium dark level. Next, the coffee beans are mashed using a grinder machine until a powder is obtained that passes sieve number 40 (Prasasti et al., 2023).
3. Extract Making: Finely ground coffee beans are put into maceration jars, given 70% ethanol solvent, with a solvent and simplicia ratio of 1:4. Soaked for 3 x 24 hours, with occasional stirring. Then it is filtered to separate the filtrate and the residue, using filter paper. The filtrate is evaporated using a vacuum drying machine with a temperature of no more than 600C until a thick extract is obtained (Prasasti et al., 2024).
4. Extract Quality Parameters
    a) Organoleptic: Organoleptic examination by observing the shape, color, and smell of coffee bean extract (Kusriani et al., 2023).
    b) pH: The pH measurement of coffee bean extract is carried out with a pH meter calibrated using pH buffers of 4.0 and 7.0. A total of 1 gram of extract is diluted with 100 mL of aquadest, then the probe is dipped until it shows a stable pH value (Chandra et al., 2019).

c) Moisture Content: The moisture content of robusta coffee bean extract is determined using Karl Fischer Titrator (Metrohm 870 KF Titrino Plus). A total of ±50 mg of samples were put into a titration chamber containing CombiMethanol-5 solvent, then the moisture content results were automatically displayed as a percentage (% w/w) of the sample weight (Ministry of Health, 2000).

5. Phytochemical Screening Testing
   a) Alkaloid Identification: A total of 0.5 g of extract was dissolved in 1 mL of HCl 2 N and 9 mL of aquades, then 2 drops of Dragendorff reagent were added and observed for 30 minutes. Positive results of alkaloids are indicated by the formation of orange deposits.
   b) Flavonoid Identification: As much as 0.5 g of the extract is dissolved in 5 mL of hot water, heated for 5 minutes, then filtered. A total of 2 mL of filtrate is added 0.1 g of Mg powder and 1 mL of concentrated HCl, then stirred. A positive result of flavonoids is indicated by the appearance of red, yellow, or orange colors.
   c) Saponin Identification: As much as 1 g of the extract is dissolved in 10 mL of hot water, heated for 5 minutes, then filtered. A total of 5 mL of cold filtrate is shaken vigorously for 10 seconds. The positive result of saponins is indicated by the formation of a stable foam 1–10 cm high that does not disappear after the addition of 1 drop of HCl 2 N.
   d) Tannin Identification: A total of 0.5 g of extract is dissolved in 10 mL of aqueducts, filtered, and filtrate diluted until colorless. A total of 2 mL of solution is then added 1–2 drops of $FeCl_3$ 1%. Positive tannin results are indicated by a change in color to green, blue, or blackish.
   e) Polyphenol Identification: A total of 0.5 g of extract is dissolved in 10 mL of distilled and filtered water. The filtrate is mixed with 10 mL of hot aquades, cooled, then 3–4 drops of 10% NaCl and a solution of $FeCl_3$ are added. The change in color from bluish-green to black shows positive results in the presence of polyphenol compounds (Wulandari et al., 2023).
6. Preparation of Test Animals: The test animals used were 25 male white rats (Rattus norvegicus) of the Sprague Dawley strain in healthy condition, active based on visual observations, and did not show anatomical abnormalities. The rats are 3–4 months old and weigh 170–200 grams. The study was divided into five treatment groups with five mice each, according to Federer's formula (Ginting et al., 2022).

   Information:
   t = Number of treatments
   n = Number of reps required

   So $(n-1)(t-1) \geq 15$
   $(n-1)(5-1) \geq 15$
   $4(n-1) \geq 15$
   $4n \geq 19$
   $N \geq 4.75 \sim 5$

   For seven days, the mice were acclimatized to adjust to the cage environment and minimize stress so that they could express their natural behavior. Each group of rats was kept in five cages according to their group, each in the form of a plastic cage measuring 80 cm^2 and 15 cm high (BPOM, 2014), with a wooden shavings base that was dried in the sun and

disinfected using 0.5% NaOCl to absorb dirt. BR-2 standard feed and boiled water are given daily ad libitum (Ollu et al., 2019).

7. Slicing Wounds in Experimental Animals: The day before the incision wound is made, the fur on the back area of the rat is shaved using an animal fur cleaner. Rats were anesthetized with Ketamine HCl at a dose of 80 mg/kg BB via intraperitoneal injection (Noor et al., 2022). The scalpel number 11 was sterilized with 70% ethanol, then the back area of the rat was cleaned using an alcohol swab after the rat lost consciousness. A 2 cm long cut was made on the back of a rat using a sterile scalpel (Rusnedy et al., 2023).
8. Wound Treatment: Wound care is carried out by administering povidone iodine and a thick extract of robusta coffee beans as much as 0.5 mL once daily for 14 days (Lafonda et al., 2025). The treatment group consisted of Group I (negative control, wound left untreated), Group II (positive control, given 10% povidone iodine), Group III (10% coffee bean extract), Group IV (20% coffee bean extract), and Group V (30% coffee bean extract).
9. Macroscopic and Microscopic Examination: Wound observation was carried out macroscopically for 14 days by assessing healing time, the presence of local infection, and allergic reactions in the wound area (Hasibuan et al., 2023). Microscopic examination was carried out through histopathological analysis by taking skin tissue from the center of the wound measuring ±3 × 2 cm. Before retrieval, the animal is anesthetized using ketamine HCl at a dose of 80 mg/kg BB, then the wound tissue is cut transversely and fixed in a 10% Neutral Buffered Formalin solution. The epidermal layer and fibroblasts were observed using Hematoxylin-Eosin staining, while collagen was analyzed with Masson Trichrome staining (Zonia, 2023).

Table 1. Scoring Nagaoka

Parameters and Description	Score
Wound healing time	
- Under 7 days	3
- Between 7- 14 days	2
- Above 14 days	1
Local infection	
- No infection	3
- Local infection with pus	2
- Local infection without pus	1
Allergic reactions	
- No allergic reactions	3
- Local allergic reaction in the form of red spots around the wound	1

10. Data Analysis: The data obtained included wound healing time, healing time score, allergic reactions, local infections, as well as epidermal count, fibroblasts, and collagen density. The wound parameter score was calculated according to Nagaoka criteria, while histopathological data was directly input into SPSS. Normality and homogeneity were tested with Shapiro-Wilk and Levene's Test, normally distributed data and homogeneous variance were analyzed using One-Way ANOVA and Post Hoc Bonferroni, while abnormal or non-homogeneous variance data were analyzed using the Kruskal-Wallis and Mann-Whitney test.

RESULTS AND DISCUSSION

Determination of Coffee Plants

Plant determination is carried out first to ensure the authenticity and accuracy of the sample species before the study continues (Ismaurasi et al., 2024). The results showed that the plant belonged to the family Rubiaceae and was identified as Coffea canephora Pierre ex A. Froehner.

Coffee Bean Extraction

Robusta coffee bean extract is obtained by maceration method using 70% ethanol solvent for 3×24 hours. Maceration was chosen because it is carried out at room temperature without heating, so that it is able to maintain the stability of thermolabile bioactive compounds such as caffeine, chlorogenic acid, and phenolic compounds (Sholikhati et al., 2023). The use of 70% ethanol is based on its water content which allows optimal penetration of cell components, thereby increasing extraction efficiency (Krisyanella et al., 2022). The extraction results produced a yield of 17.05%, meeting the general requirements (>10%) according to the Ministry of Health of the Republic of Indonesia (2000). This value shows that the extraction process takes place optimally in attracting bioactive compounds, which is influenced by the characteristics of the solvent, the content of the active compounds, and the extraction method used (Alfauzi et al., 2022).

Table 2. Yield Results of Robusta Coffee Bean Extract

Name Simplisia	Weight Simplification (gram)	Weight of Thick Extract (grams)	Rendemen (%)	Conclusion
Coffea canephora	4.450	758,8	17,05	Meet the requirements (>10%, Ministry of Health 2000)

Extract Quality Parameters

a. Organoleptic: The results of organoleptic tests show that the thick extract of robusta coffee beans has a thick consistency, blackish-brown in color, and has a distinctive coffee flavor.
b. Moisture Content: Moisture content is an important indicator of extract quality, as high levels can trigger the growth of microorganisms and lower stability. The moisture content of robusta coffee bean extract of 10.28% is still below the maximum limit of SNI 01-2907-2008 which is 12.5%, indicating that the drying and storage process of raw materials has been optimized so that the quality and stability of the extract are maintained (Nugraha et al., 2025).
c. pH: The test results show that the pH of robusta coffee bean extract is 5.21, still within the physiological range of healthy skin (pH 4.1–5.8), making it safe for topical use. The pH value supports the wound healing process, because alkaline pH can inhibit tissue regeneration, prolong the inflammatory phase, and increase the risk of infection. Variations in pH values are thought to be influenced by particle size as well as the content of organic acids, such as chlorogenic acid and acetic acid, in the extract (Rosca et al., 2025).

Screening Phytochemistry Test

Phytochemical screening was carried out to identify the content of secondary metabolite compounds in robusta coffee bean extract (Coffea canephora) (Arifah et al., 2024). Based on the test results in Table 3, the condensed extract of robusta coffee beans is known to contain alkaloids, flavonoids, saponins, tannins, and polyphenols.

Table 3. Robusta Coffee Bean Extract Phytochemical Screening Test Results

Metabolite Seconds	Reagents	Parameter	Test Results
Alkaloid	HCl 2 N + Dragendorff	Orange deposits are formed	Positive (+)
Flavonoid	Concentrated Mg+HCl powder	Discoloration from red to orange	Positive (+)
Saponins	Shake + HCl 2 N	Stable foam is formed 1–10 cm high that lasts a long time	Positive (+)
Tannins	$FeCl_3$	Color change from green to blackish	Positive (+)
Polyphenols	NaCl + $FeCl_3$	Blackish-green discoloration	Positive (+)

The secondary metabolites in robusta coffee bean extract play an important role in wound healing through various biological mechanisms. Alkaloids are antibacterial, anti-inflammatory, and help vasoconstriction in the hemostasis phase. Flavonoids and tannins function as antimicrobial, anti-inflammatory, and have an astringent effect that accelerates wound closure and reduces inflammation. Saponins stimulate the formation of new tissues and increase collagen synthesis during the proliferation phase (Carolina et al., 2022). In addition, polyphenols, especially chlorogenic acid and caffeine, have high antioxidant activity that protects tissues from oxidative stress (ROS) and stimulates collagen regeneration, thereby accelerating the wound healing process (Elfiah et al., 2023).

Test the Effectiveness of Robusta Coffee Bean Extract in Healing Cut Wounds

Macroscopic Examination of Cut Wounds

The wound healing process progresses progressively through four phases, namely hemostasis, inflammation, proliferation, and remodelling or maturation, The hemostasis phase (day 0–1) is characterized by platelet activation and the formation of blood clots that form scabs to stop bleeding, protect the wound, and maintain tissue moisture (Ricardo et al., 2024). The inflammatory phase (days 1–3) is characterized by redness, slight swelling, and exudate (Sukma et al., 2025). In the robusta coffee bean extract treatment group, the wound appeared to dry faster accompanied by the formation of thick scabs, signaling a transition to the early proliferation phase (Aulia et al., 2024). The proliferation phase (day 4–10) is characterized by narrowing of the wound area, granulation of tissue, epithelialization, and angiogenesis that play an important role in tissue regeneration (Malaha et al., 2023). The remodelling or maturation phase (days 10–14) is characterized by the removal of the scab and the formation of a new layer of the epidermis. In the tissues, there is a process of collagen reformation, a decrease in the number of fibroblast cells, and the formation of stronger and more stable scar tissue. In the 20% and 30% treatment groups, scabs began to come off faster than the 10% group, indicating a more optimal wound healing process (Salim et al., 2024).

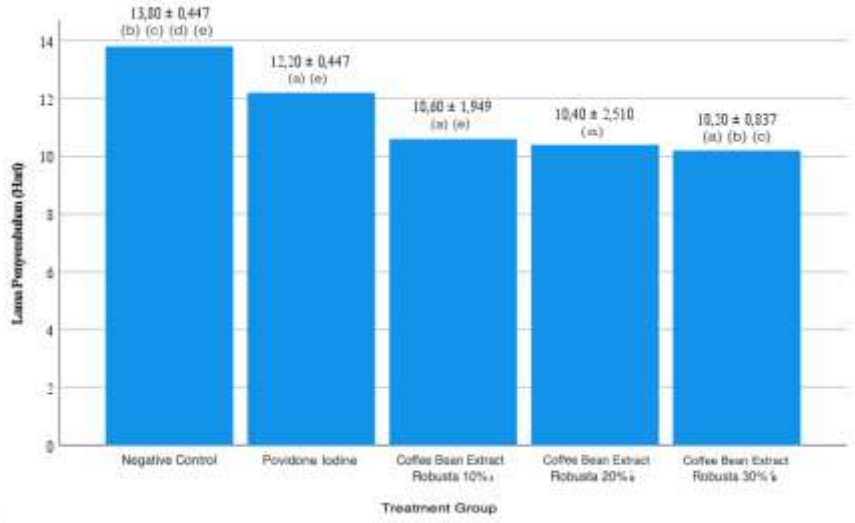

Figure 1. Old Graph of Cut Wound Healing

Remarks: Significant differences ($p < 0.05$) between groups (a) Negative control (b) Positive control, (c) 10% robusta coffee bean extract, (d) 20% robusta coffee bean extract, (e) 30% robusta coffee bean extract.

Based on Figure 1, the negative control group showed the longest cure time (13.80 days), while the positive control was 12.20 days. The treatment group with robusta coffee bean extract concentrations of 10%, 20%, and 30% showed a faster cure time, 10.60 respectively; 10,40; and 10.20 days. This is in line with the research of Alphama et al. (2019), which showed that the use of povidone iodine was not significantly more effective than the robusta coffee bean powder group in accelerating wound healing based on an average time of 10.56 days. The results of the Kruskal–Wallis analysis showed significant differences between groups ($p = 0.016$), and the Mann–Whitney test confirmed that the extract was 30% significantly different from the negative control, positive control, and 10% ($p = 0.007$). Robusta coffee bean extracts with concentrations of 20% and 30% have been shown to be most effective in accelerating wound healing, allegedly due to the content of chlorogenic acid, caffeine, flavonoids, tannins, and phenolic acids that are antioxidant, anti-inflammatory, and antibacterial, thereby accelerating tissue regeneration and preventing infection (Santos et al., 2024).

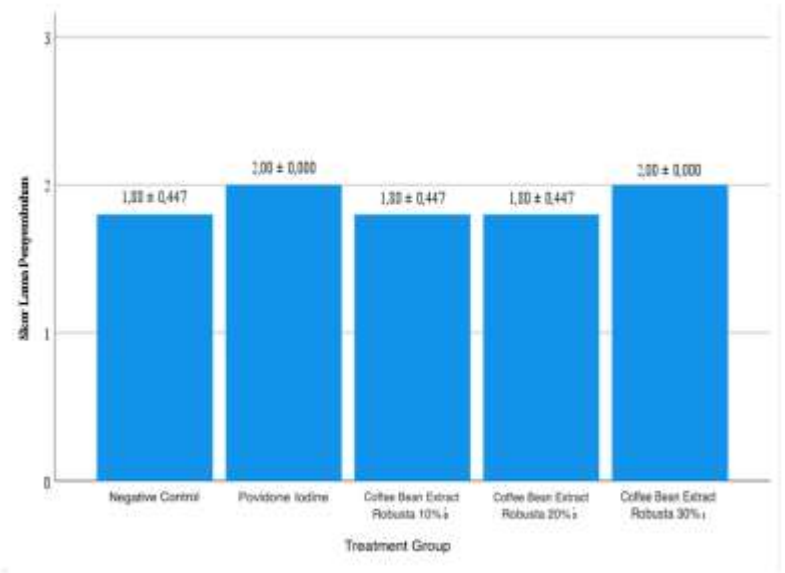

Figure 2. Scissors Healing Old Score Chart

Based on Nagaoka scoring on wound healing time, which can be seen in Figure 2, the lowest average values were obtained in the negative control group as well as the 10% and 20% robusta coffee bean extract treatments. This condition was caused by one test animal that had not experienced optimal healing until the 14th day. These variations are thought to be influenced by biological factors such as immunity, psychological conditions, and the environment that play a role in the speed of the wound healing process (Dini et al., 2022). Statistical analysis with the Kruskal-Wallis test showed no significant difference between all groups p = 0.702 (p>0.05).

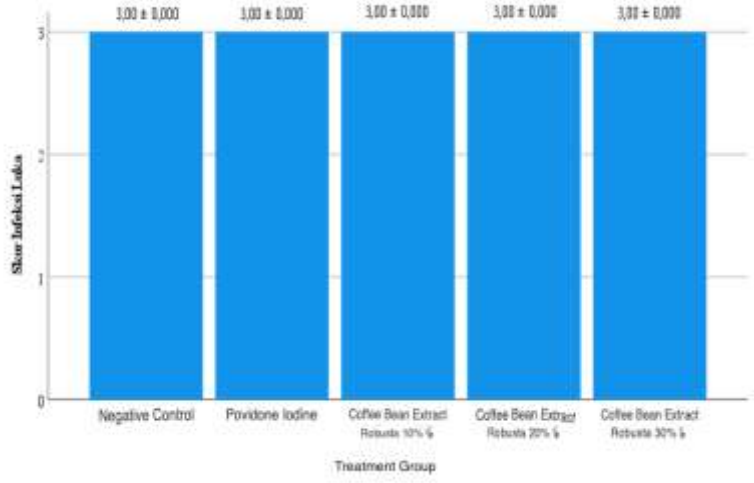

Figure 3. Cut Wound Infection Score Chart

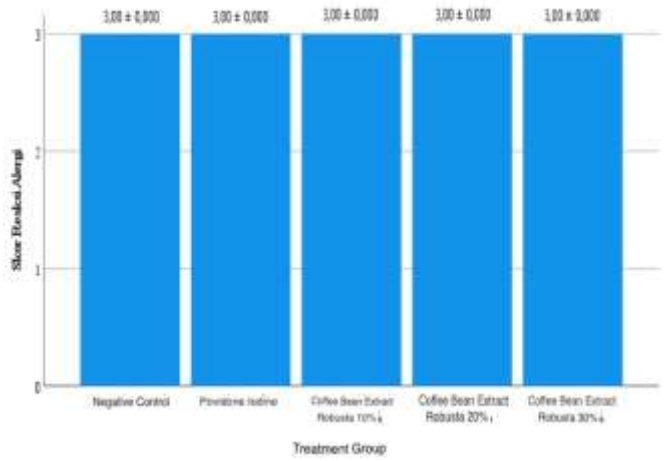

Figure 4. Cut Wound Allergic Reaction Score Chart

Based on Nagaoka scoring on wound infection scores and allergic reactions shown in Figure 3 and Figure 4, the entire group, both negative control, positive control, and treatment with robusta coffee bean extract concentrations of 10%, 20%, and 30%, showed the same values (3.00 ± 0.000). The score value of 3 indicates that there are no signs of infection and allergic reactions to the wound, so it can be concluded that the administration of robusta coffee bean extract does not cause infectious reactions and allergic reactions to the injured skin so it is safe to use. One of the important factors in wound healing is infection control. Robusta coffee bean extract contains active compounds such as chlorogenic acid, caffeine, trigonelin, and flavonoids, which have antibacterial activity against various pathogens, including Methicillin Resistant Starhylococcus Aureus (MRSA) (Febiaocti et al., 2025).

Microscopic Examination of Cut Wounds

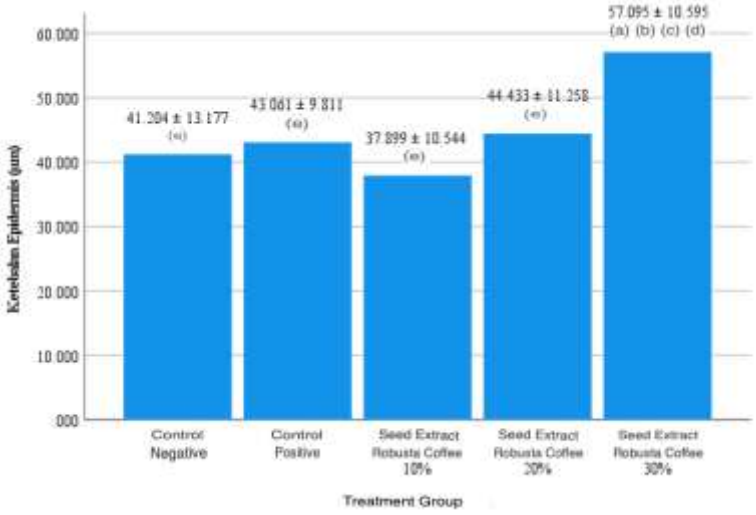

Figure 5. Average and Standard Deviation of Epidermal Thickness

Remarks: Significant differences ($p < 0.05$) between groups (a) Negative control, (b) Positive control, (c) 10% robusta coffee bean extract, (d) 20% robusta coffee bean extract, (e) 30% robusta coffee bean extract

Based on Figure 5, the thickness of the epidermis increases with the increase in the concentration of robusta coffee bean extract, with the highest value at a concentration of 30%. This improvement suggests that robusta coffee bean extract plays a role in accelerating the regeneration of skin tissue through the activity of its bioactive compounds. The content of triterpene saponins is known to stimulate the proliferation of epithelial cells, while tannins accelerate the reepithelial process, thereby supporting the formation of new epidermal layers (Elfiah et al., 2023). The thickness of the epidermis is an indicator of healing success, where the thicker the layer formed indicates a more optimal tissue regeneration process (Amfotis et al., 2022). The results of the Kruskal–Wallis test showed a significant difference between groups ($p = 0.013$), and the Mann–Whitney follow-up test showed that the 30% extract group was significantly different ($p < 0.05$) compared to the other groups, suggesting that the concentrations of 20% and 30% were effective in increasing the thickness of the epidermis during the wound healing process.

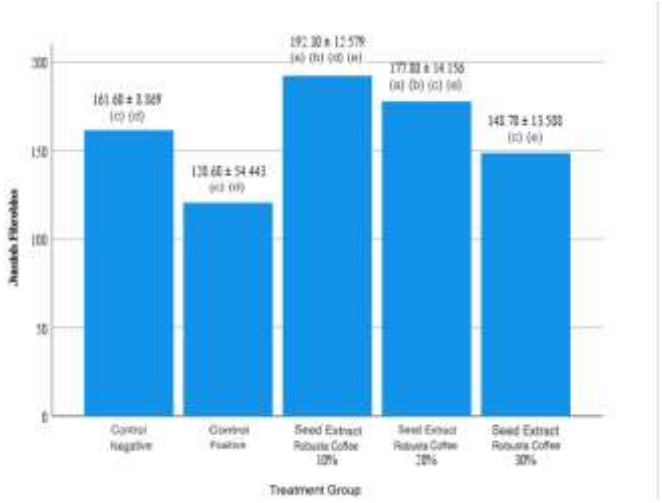

Figure 6. Mean and Standard Deviation of Fibroblast Count

Remarks: Significant differences ($p < 0.05$) between groups (a) Negative control (wound left untreated), (b) Positive control (povidone iodine 10%), (c) 10% robusta coffee bean extract, (d) 20% robusta coffee bean extract, (e) 30% robusta coffee bean extract.

An examination of the number of fibroblasts can be seen in Figure 6, showing that the 10% robusta coffee bean extract group had the highest number compared to the other groups, indicating that the concentration was able to stimulate the proliferation of fibroblasts optimally in the early healing phase. This activity is related to the role of bioactive compounds such as chlorogenic acid, flavonoids, tannins, and caffeine which are antioxidant and anti-inflammatory, so that they are able to stimulate the formation of granulated tissue and accelerate skin regeneration (Yuwono et al., 2022). A decrease in the number of fibroblasts at concentrations of 20% and 30% indicates that the healing process has entered a remodelling phase, in which fibroblast activity decreases physiologically through apoptosis mechanisms to prevent the formation of excess connective tissue (Cialdai et al., 2022). In addition, at higher concentrations of extracts, there is a possibility of a ceiling effect, which is a condition when an increased dose no longer provides a therapeutic effect, and can even inhibit the cellular response or cause the opposite effect (Rahmadani et al., 2022). Statistical analysis using the Kruskal–Wallis test showed significant differences between groups ($p = 0.000$), and the Mann–

Whitney follow-up test confirmed the effect of variation in extract concentration on the number of fibroblasts ($p < 0.05$).

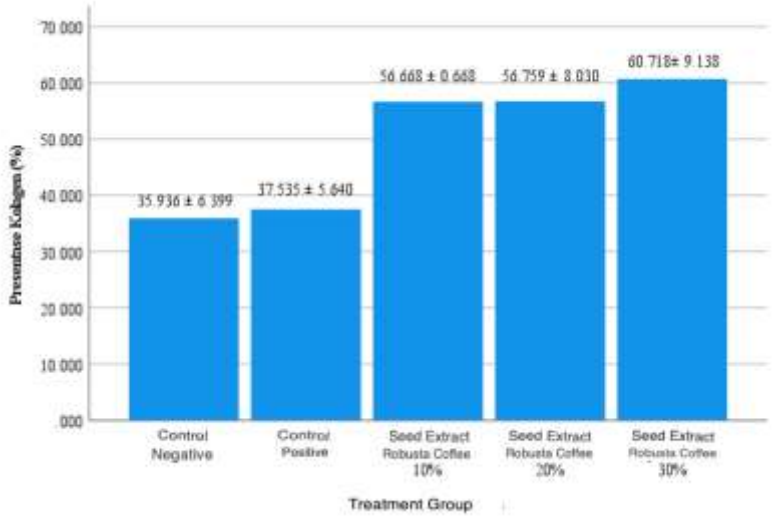

Figure 7. Average and Standard Deviation of Collagen Amount

Collagen is the main component of the extracellular matrix that plays a role in the proliferation phase to the maturation of wound healing. Its formation begins from the 3rd day to the second week, starting with type III collagen which is then replaced by type I to strengthen new tissues (Suryadewi et al., 2023). Based on the results of the study in Figure 7, the negative and positive control groups showed lower collagen levels than the treatment group. This is suspected to be due to the use of povidone iodine which can inhibit the growth of fibroblasts so that collagen synthesis decreases and can slow down wound healing (Llamas et al., 2022). In contrast, increased collagen levels in the treatment group showed a positive effect of robusta coffee bean extract on the formation of new tissues. This effect is related to the content of chlorogenic acids, flavonoids, and saponins that are anti-inflammatory and antioxidant. Chlorogenic acids and flavonoids inhibit the enzymes cyclooxygenase and lyoxyglycenase, degrade prostaglandins and leukotrienes, and accelerate the transition from the inflammatory phase to proliferation. These two compounds also increase the secretion of TGF-β through macrophage activation, while saponins strengthen the expression of TGF-β receptors on fibroblasts and stimulate fibronectin synthesis. The synergistic effects of these three compounds increase fibroblast activity, collagen synthesis, and accelerate wound healing (Aisyah et al., 2023).

CONCLUSION

Robusta coffee bean extract (Coffea canephora) at concentrations of 20% and 30% demonstrated the most effective acceleration of cut wound healing and significant improvement in tissue histopathology in male Sprague Dawley rats, marked by enhanced epidermal layer restoration, increased fibroblast proliferation, and greater collagen formation. Future research should explore the molecular mechanisms underlying these effects and assess

the extract's efficacy and safety in clinical trials to confirm its therapeutic potential in human wound care.

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