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Evaluating the synergistic antibacterial and anti-inflammatory effects of Kapok banana peel (Musa acuminata balbisiana colla) and Bay leaf (Syzygium polyanthum) extract gel formulation on wound healing

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Abstract: The body's natural healing mechanisms rely significantly on substances that enhance tissue regeneration. Rapid growth of new skin cells can considerably accelerate wound healing. This study investigates the effectiveness of gel formulations derived from Saba banana peel (*Musa acuminata balbisiana Colla*) and bay leaf (*Syzygium polyanthum*) extracts as anti-inflammatory and antiseptic agents. Utilizing a true-experimental posttest-only control group design, we evaluated three different gel formulations: F1 (25% banana peel extract : 75% bay leaf extract), F2 (50% banana peel extract : 50% bay leaf extract), and F3 (75% banana peel extract : 25% bay leaf extract). Twenty white rats (*Rattus norvegicus*) were divided into five groups, each subjected to standardized incision wounds. Treatments were administered three times daily over a seven-day observation period, with wound healing assessed by measuring incision length. Formulation F1 demonstrated notable antibacterial efficacy against *Staphylococcus aureus.* Conversely, formulation F3 (75% banana peel : 25% bay leaf) exhibited superior wound healing performance, achieving an average healing rate of 42.97%, surpassing the positive control group. These findings indicate that gels containing extracts of Saba banana peel and bay leaf possess significant antibacterial activity against *S. aureus* and effectively enhance wound healing in animal models.

Keywords: Anti-inflammatory; Antibacterial; Bay Leaf; Gel; Saba Banana Peel.

INTRODUCTION

Incidences of cuts or incisions occur frequently in both medical and non-medical contexts worldwide. In developing countries, the incidence tends to be higher due to increased injury risks, substandard medical practices, and inadequate sanitation conditions. Conversely, in developed countries, intentional surgical incisions are commonly performed in procedures such as general surgery, cardiac surgery, and orthopedic surgery. Global data indicate that annually there are approximately 110.3 million surgical wounds, 1.6 million incision wounds, 20.4 million abrasions, 10 million burns, 8.5 million pressure ulcers, 12.5 million venous ulcers, 13.5 million diabetic ulcers, 200,000 amputations, 600,000 wounds related to cancer, 100,000 cases of malignant melanoma, and 100,000 cases involving complications from skin cancer[1].

In Indonesia, the prevalence of injuries remains significantly high, with recent data indicating a national prevalence rate of 8.2%, an increase of 0.7% compared to the previously reported 7.5% five years earlier. Cuts or incisions

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constitute the second most common type of injury, following scratches or bruises primarily caused by falls, which account for approximately 70.9% of all reported injuries. Specifically, cuts and tears represent 25.4% and 23.2% of total injuries, respectively[2].

The risk of bacterial infections, particularly involving Methicillin-Resistant Staphylococcus aureus (MRSA), poses significant challenges in wound management due to growing antibiotic resistance. Consequently, implementing effective management strategies is essential to prevent infections and promote rapid wound healing. The use of antibacterial and anti-inflammatory agents is standard practice aimed at eliminating pathogenic microorganisms and minimizing inflammation[3].

Povidone iodine is commonly utilized in wound care because of its broad antimicrobial efficacy and capacity to create a moisture-supportive environment that supports angiogenesis. However, in vitro studies have shown that povidone iodine at concentrations of 10% may inhibit fibroblast proliferation, a crucial process in wound healing. Additionally, povidone iodine can cause adverse reactions such as iododerma, chemical burns, and anaphylactic reactions[4].

Natural alternatives, such as banana peel and bay leaf extracts, have individually demonstrated promising antibacterial and anti-inflammatory properties. Bay leaves (*Syzygium polyanthum*) contain essential oils rich in phenolic compounds, notably chavicol, which exhibits antibacterial activity five times greater than phenol. Eugenol, another key component found in bay leaves, has recognized anti-inflammatory, antibacterial, and analgesic effects that support wound healing processes[5]. Similarly, banana peels (*Musa acuminata balbisiana Colla)* possess pharmacological potential, with bioactive compounds that exert anti-inflammatory activity by inhibiting Reactive Oxygen Species (ROS), protecting protease inhibitors from oxidative damage, and preventing fibroblast degradation. Additionally, banana peels exhibit antibacterial effects through mechanisms that inhibit bacterial enzyme activity and nucleic acid synthesis[6].

Despite substantial research on banana peel and bay leaf extracts individually, there remains a significant research gap regarding their combined synergistic effects in wound-healing formulations. Addressing this gap is particularly critical given the increasing demand for effective, low-toxicity, and affordable natural treatments for wound care, especially in resource-limited and antibiotic-resistant contexts. Therefore, this study aims to investigate the synergistic antibacterial and anti-inflammatory properties of combined extracts from kapok banana peel (*Musa acuminata balbisiana Colla*) and bay leaf (*Syzygium polyanthum*) formulated into a topical gel. Specifically, this research will evaluate the efficacy of the gel formulation in accelerating wound healing in incision wounds using male Wistar rats (*Rattus norvegicus*).

MATERIALS AND RESEARCH METHOD

Research design

This study employed a true experimental design with a posttest-only control group. The research was conducted from July to October 2023 in the Pharmacology, Pharmaceutical Chemistry, Pharmaceutical Biology, and Microbiology laboratories at Universitas Muhammadiyah Purwokerto.

The anti-inflammatory properties of the gel formulated from kapok banana peel and bay leaf extracts were evaluated using 20 male Wistar rats randomly divided into five groups (n=4 per group): formulation F1 (25% banana peel extract:75% bay leaf extract), formulation F2 (50% banana peel extract:50% bay leaf extract), formulation F3 (75% banana peel extract:25% bay leaf extract), a positive control group (K+) treated with Bioplacenton, and a negative control group (K-) without treatment. Each rat received an incision wound and was treated with the respective gel three times daily for seven days. The incision length was measured daily to assess wound healing progression.

The antibacterial properties of the gel were evaluated against *Staphylococcus aureus* using four replicates for each treatment group: formulations F1, F2, and F3, with chloramphenicol as the positive control (K+) and 5% dimethyl sulfoxide (DMSO) as the negative control (K-). The formulation ratios were selected based on preliminary findings that indicated optimal antibacterial and anti-inflammatory efficacy. Ethical approval was granted by the Health Research Ethics Commission of Universitas Muhammadiyah Purwokerto (Approval number: KEPK/UMP/64/VIII/2023).

Materials and equipment

Materials used included bay leaf (*Syzygium polyanthum*, locally sourced), kapok banana peel (*Musa acuminata balbisiana Colla*, organic farms), 70% ethanol (Merck, 99% purity), ether (Sigma-Aldrich, 99.5% purity), hydrochloric acid (Fisher Chemical, 37% purity), ethanol 96% (VWR International, 99.9% purity), 6.5% carbonate solution, triethanolamine (TEA, Acros Organics, 99% purity), methylparaben (Alfa Aesar, 99% purity), glycerin (Avantor Performance Materials, 99.5% purity), distilled water, propylene glycol (Sigma-Aldrich, 99.5% purity), diffusion paper, soap, chloramphenicol (TCI Chemicals, 98% purity), and Bioplacenton.

Equipment utilized included an analytical balance (accuracy 0.0001 g), drying cabinet, grinder, Büchner funnel, vacuum rotary evaporator, water bath, UV-Vis spectrophotometer (UV-1800, wavelength range 190–1100 nm), Fourier-transform infrared (FTIR) spectrophotometer (IR Affinity-1, resolution 0.5 cm^{-1}), pH indicator strips (range 0–14), measuring cylinders (50 mL), Brookfield RVT viscometer (spindle LV, 100 rpm), round glass petri dishes (15 cm diameter), transparent glass plates, and sterile cotton.

Preparation and extraction

Bay leaves and kapok banana peels were cleaned, weighed, dried either by oven or sunlight, and ground into a fine powder. Extraction was performed using maceration with 96% ethanol to isolate bioactive compounds such as flavonoids, tannins, and saponins. The resulting filtrate, obtained by filtration through a Büchner funnel, was concentrated using a rotary evaporator at 70°C, followed by further evaporation in a water bath at 50°C to yield a concentrated extract. The extracts were subsequently diluted to concentrations of 25%, 50%, and 75%.

Identification of main compounds

Fourier-transform infrared spectroscopy (FTIR) was utilized to identify secondary metabolites and functional groups present in the extracts by analyzing infrared absorption spectra[7].

Gel formulation

Gel formulations were prepared by thoroughly mixing gel base components with the respective extracts. The detailed compositions for formulations and control groups are presented in Table 1.

Table 1. Composition of Antibacterial and Anti-inflammatory Gel

Material	K-	K+	F1	F2	F3
Saba Banana Peel:Bay Leaf (g)	-	-	25%:75%	50%:50%	75%:25%
TEA (g)	3	-	3	3	3
Methyl paraben (g)	0,09	-	0,09	0,09	0,09
Glycerin (g)	6	-	6	6	6
Propylene glycol (g)	0,15		0,15	0,15	0,15
Aquadest (mL)	100	-	100	100	100
Bioplasenton (g)	-	15	-	-	-

K-: Negative Control (w/w%)
K+: Positive Control (w/w%)
F1: Formulation 1 (w/w%)
F2: Formulation 2 (w/w%)
F3: Formulation 3 (w/w%)

Gel evaluation

Gels were evaluated for pH, viscosity, homogeneity, spreadability, and adhesion based on Indonesian National Standards (SNI 16-4380-1996). Stability tests were conducted at temperatures of 45°C and 4°C to assess durability under different storage conditions. Acceptable pH ranged from 4.5 to 6.5, while viscosity was measured using a Brookfield RVT viscometer, with the acceptable range between 3,000 and 50,000 cP[8].

Antibacterial testing

Antibacterial activity against *S. aureus* was assessed using the disc diffusion method. Nutrient agar plates were inoculated with a standardized bacterial suspension adjusted to a McFarland 0.5 standard (approximately 1×10^7 to 1×10^8 CFU/mL). Paper discs soaked in gel samples were placed on inoculated plates and incubated at 37°C for 24 hours. Zones of inhibition were measured using calipers[9].

Anti-inflammatory testing

Male Wistar rats (*Rattus norvegicus*, 150–180 g) were acclimated for one day. Anesthesia was induced using ether inhalation, after which a 3-cm dorsal incision wound of subcutaneous depth was inflicted following shaving of the area. Each wound was treated with 1 g of gel applied three times daily using sterile cotton. Observations on wound healing progression were made over 14 days, evaluating macroscopic healing and reductions in wound size[11].

Statistical analysis

Data were analyzed statistically using Kruskal-Wallis tests followed by Mann-Whitney U tests for pairwise comparisons. Statistical significance was determined at $p<0.05$.

RESULTS AND DISCUSSION

Fourier Transformed Infrared (FTIR) is an instrument that can be used to identify functional groups, detect compounds, especially organic compounds, and analyze mixtures from samples without damaging them. The range of the electromagnetic spectrum of the infrared spans from 14000 cm^{-1} to 10^{-1}. To analyze the functional groups in a sample, the absorption bands that appear in the infrared spectrum are compared with the spectrum of known reference compounds (as listed in the IR table) [7]. The FTIR spectra of Saba banana peel and Bay leaf extracts (Figure 1) reveal peaks corresponding to tannin, saponin, and flavonoid compounds, which are associated with antibacterial and anti-inflammatory properties.

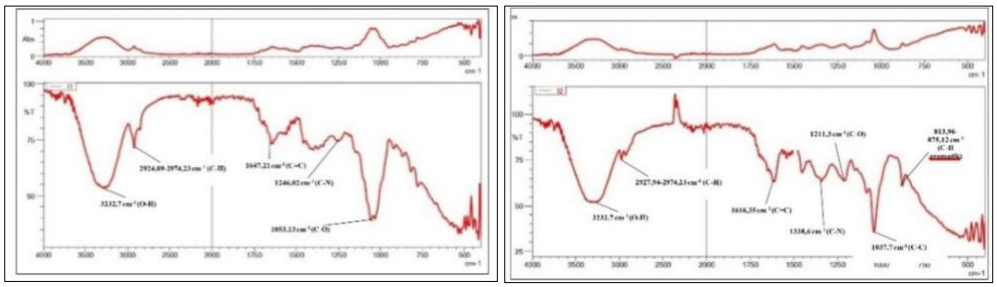

Figure 1. FTIR Analysis of Saba Banana Peel Extract (right) and Bay Leaf Extract (left)

Table 2. FTIR Spectrum Analysis Results

No	Group	Compound Derivative / Functional Group	
		Saba Banana Peel Extract	**Bay Leaf Extract**
1	Flavonoid	O-H (*phenol*), C-H (*aglycone*), C=C (*aromatic ring*),	O-H (*phenol*), C-H (*alkane*), C=C (*aromatic ring*)
2	Tanin	O-H (*phenol*), C-H (*aromatic*)	O-H (*phenol*), C-H (*aromatic*)
3	Saponin	C-N (*amine*), C-O (*ether*)	C-N (*amine*), C-O (*ether*)

Based on the FTIR spectrum analysis results (Table 2) of the kapok banana peel extract and bay leaf extract, functional groups of compounds such as tannin, flavonoid, and saponin were identified, which have the potential to act as antibacterial and anti-inflammatory compounds. The bioactive compounds identified include ascorbic acid glucoside, methyl salicylate, linalool, and betaine (Table 3). These compounds are critical for antibacterial activity by disrupting bacterial membranes and inhibiting essential metabolic pathways. The anti-inflammatory properties are mediated through flavonoids, which modulate inflammatory mediators and promote wound healing by enhancing fibroblast proliferation and collagen synthesis.

Table 3. Main Compound Components

No	Score	Saba Banana Peel Extract	Score	Bay Leaf Extract
1	603	*A_Methyl Salicylate*-4	583	*A_Methyl Salicylate*-4
2	597	*A_L-Ascorbic Acid 2-Glucoside*-4	577	*A_Linalool*-4
3	-	-	573	*A_Betaine*-4

Ascorbic acid glucoside is a compound that is a derivative of vitamin C. Vitamin C has been studied for its potential antibacterial activity against several types of bacteria, although ascorbic acid glucoside itself may have a weaker antibacterial effect compared to pure vitamin C. *A_L-Ascorbic Acid 2-Glucoside* (AA2G) has been shown to have antibacterial properties and can protect cells from early DNA damage, providing protection against radiation and bacterial infection, particularly *S. aureus* [12]. AA2G has been proven to have anti-inflammatory properties that can accelerate wound healing by promoting angiogenesis (formation of new blood vessels) and collagen deposition [13].

Methyl salicylate is a compound derived from salicylic acid and can be used as a topical anti-inflammatory that works by inhibiting the production of prostaglandins, which are the main mediators of inflammation in the body. *Methyl salicylate* has strong antibacterial properties, especially against gram-positive bacteria such as *S. aureus* and *Streptococcus pneumoniae* [14].

Linalool is a compound found in essential oils. *Linalool* has been shown to have antibacterial activity against various types of bacteria, such as *S. aureus* and *Escherichia coli*. This is due to its ability to damage the bacterial cell membrane and inhibit microbial growth, thereby preventing bacterial infection in wounds. *Linalool* has demonstrated anti-inflammatory properties, especially in reducing the production of inflammatory mediators such as cytokines and prostaglandins, and inhibiting the activity of enzymes involved in the inflammatory response [15].

Betaine has shown antibacterial activity against various types of bacteria, including *S. aureus* and *Salmonella spp*. It can disrupt the integrity of the bacterial cell membrane and inhibit bacterial growth. *Betaine* has weak anti-inflammatory properties; however, it is capable of suppressing the production of pro-inflammatory cytokines and inhibiting the inflammatory response in wounds [16].

Based on the physicochemical characteristics (Table 4), all gel samples (F1, F2, and F3) show no color, odor, or form changes, indicating that the gels maintain visual consistency and aroma. The pH test on the gels is conducted to determine whether a preparation is safe for use on the skin. A pH that is too low (*acidic*) can cause skin irritation, making it uncomfortable for users, while a high pH can cause the skin to dry out [17]. Sample F1 tends to be more acidic compared to samples F2 and F3. The pH test results prove that the gel pH still falls within the standard pH range for good skin pH. The F1 gel, with a higher bay leaf concentration, exhibited the highest viscosity, contributing to structural stability but reduced spreadability. Conversely, the F3 gel, with a higher banana peel concentration, demonstrated superior spreadability, essential for effective topical application. All samples (F1,

F2, and F3) are homogeneous, indicating that there is no significant difference between the parts of the gel samples.

Table 4. Results of Gel Evaluation Test

Gel	Organoleptic	pH (4,5-6,5)	Viscosity (3000-50000 cPs)	Homogeneity	Spreadibility (5-7 cm)	Adhesion (>1 sec)
F1	There is no change in color, odor, or change in shape	5	9460 Cps	Homogeneous	5,1	7
F2	There is no change in color, odor, or change in shape	6	4730 Cps	Homogeneous	5,8	2
F3	There is no change in color, odor, or change in shape	7	3750 Cps	Homogeneous	7	1,5

Table 5. Results of Gel Stability Test

Day	Temperature 45^{O}C	Temperature 4^{O}C
1	Stable, no changes yet	Stable for 7 days without any changes
2	Gel darkens	
3	Bacteria growth in gel F3, F2 and F3 harden	
4	Bacteria growth in gel F3, F2 and F3 harden	
5	Gels F1 and F2 start to dry, while F3 does not	
6	Gels F1 and F2 start to dry, while F3 does not	
7	F1: Gel becomes clumpy, dry F2: Gel dries out F3: Bacterial growth in the gel, same shape	

Based on the gel stability test conducted on different gel formulations, consisting of F1, F2, and F3, placed at two temperatures, 45°C and 4°C for 7 days with daily observations (Table 5). At 45°C, significant changes occurred in the gels, such as color alteration, bacterial growth, drying, and consistency changes. Gels F1 and F2 started to dry and clump, while gel F3 experienced bacterial growth and darkening. These results could be due to the high temperature, non-aseptic testing, and lack of sterilization in the storage area, leading to bacterial contamination and changes in the gels. Meanwhile, at 4°C, the results obtained for gels F1, F2, and F3 remained stable for 7 days without any changes, indicating that the optimal storage temperature for the gels is 4°C to maintain stability. Temperature plays a crucial role in influencing the properties and stability of gel formulations. At 45°C, significant degradation occurred, including bacterial contamination and drying, indicating the need for cold storage to maintain efficacy.

Table 6. Antiseptic Test Results

Treatment Group Repetition (4)	Median (Min-Max)	P Value
K+	24.2(10.4-32.3)	0.007
K-	0(0-0)	
F1	2.8(2.1-5.7)	
F2	0.85(0-6.4)	
F3	0(0-3.1)	

The *Kruskal-Wallis* analysis in Table 6 shows the significance value (sig.) of p=0.007 (p<0.05) indicates a significant difference between the treatment group and the control group in terms of inhibitory effects on *S. aureus* bacteria. The data were further tested using the *Mann-Whitney U* test to identify groups with significantly different relationships.

Table 7. *Man Withney-U* Test Results of Saba Banana Peel and Bay Leaf Extracts on *S. aureus* Bacteria

Treatment	Diameter (P Value)
K+ x K-	0.014*
F1 x K+	0.021*
F1 x K-	0.014*
F1 x F2	0.245
F1 x F3	0.139
F2 x K+	0.020*
F2 x K-	0.131
F2 x F3	0.508
F3 x K+	0.018*
F3 x K-	0.317

Note: *significant

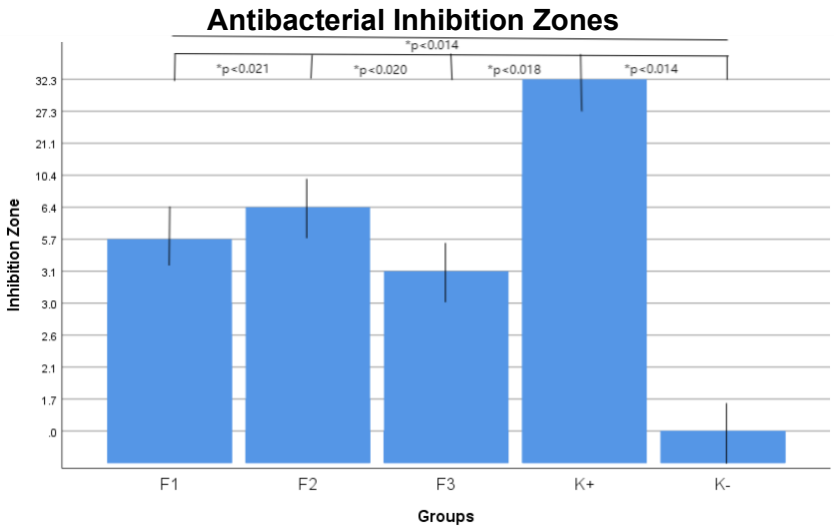

Figure 2. Antibacterial Inhibition Zones for the Different Treatments Against *S. aureus*

Significant differences (*p<0.05) are annotated directly on the Figure 2. The *Mann-Whitney U* test results in Table 7 show a significant difference in the effects of kapok banana peel and bay leaf extracts on inhibiting the growth of *S. aureus* bacteria between the K+ and K- groups, F1 and K+ groups, F1 and K- groups, F2 and K+ groups, as well as F3 and K+ groups (p<0.05).

The F1 treatment (25% Saba banana peel: 75% bay leaf) proved to be more effective in inhibiting the growth of *S. aureus* bacteria compared to the F3 treatment (75% Saba banana peel: 25% bay leaf). The superior antibacterial activity of F1 (25% banana peel: 75% bay leaf) against *S. aureus* can be attributed to the high concentration of *eugenol* and *linalool* in bay leaf extract, which disrupt bacterial cell membranes and inhibit enzyme activity., as evidenced by the increased diameter of the inhibition zone against *S. aureus* bacteria. This demonstrates that the antibacterial compounds in bay leaf have the ability to slow the growth of *S. aureus* bacteria.

The main compound in bay leaf is essential oil. Essential oil consists of hydrophobic molecules that play a role in disrupting the function of enzymes associated with the cell membrane, thus disrupting the membrane's activity and causing bacterial cell death. *S. aureus* has a main component in the form of a thick cell wall. The cell wall of *S. aureus* bacteria is composed of a single plasma membrane surrounded by peptidoglycan. Therefore, the cell wall of *S. aureus* is the main target of the active substance in the gel extract of bay leaf and Saba banana peel [18].

The antibacterial mechanism inhibits bacterial growth by inhibiting cell wall synthesis, metabolism synthesis, disrupting cell membrane integrity, inhibiting cell protein synthesis, and inhibiting nucleic acid synthesis. The antibacterial components found in bay leaf can inhibit the growth of *E. coli* and *S. aureus* bacteria by inhibiting cell wall synthesis and blocking peptidoglycan synthesis stages that will damage the bacterial cell wall and cause cell lysis. Cell lysis results in the bacteria being unable to function [19].

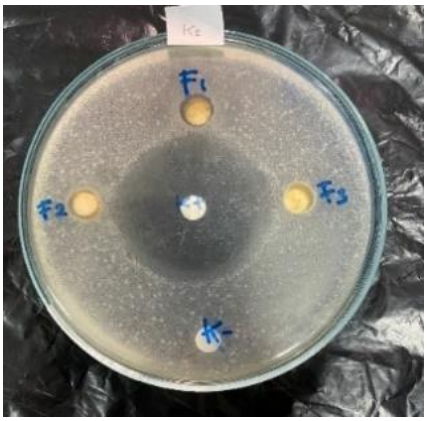

Figure 3. Results of Inhibition Zone Test of *S. aureus* Bacteria Against Gels K+, K-, F1, F2, and F3.

Based on the results of the inhibition zone test against *S. aureus* bacteria in Figure 3, it shows an average inhibition zone of 22.75 mm against the K+ gel (*chloramphenicol*), and *S. aureus* bacteria did not show an inhibition zone against the K- gel (5% DMSO). Based on observations of the diameter of the inhibition zone, the *Minimum Inhibitory Concentration* (MIC) was found at the concentration of the F1 gel with an average inhibition zone diameter of 3.4 mm against *S. aureus* bacteria. The concentration of the F2 gel could slow the growth of *S. aureus* bacteria with an average inhibition zone diameter of 2.025 mm. The concentration of F3 could slow the growth of *S. aureus* bacteria with an average inhibition zone diameter of 1 mm. The research results prove that the extracts of Saba banana peel and bay leaf have the potential to treat infections caused by *S. aureus* bacteria. The most optimal inhibition zone size was found in the F1 formulation with a concentration ratio of 50% Saba banana peel extract to 75% bay leaf extract.

Table 8. Comparison of Wound Healing Percentage in Each Treatment Group

Treatment Group Repetition (4)	Median (Min-Max)	P Value
K+	100(100-100)	0.003
K-	48.3(36,6-60,0)	
F1	49.95(43,3-73,3)	
F2	74.95(70-93)	
F3	91.50(80-100)	

The average score analysis using *the Kruskal-Wallis* test (Table 8) shows resulted in a value of P=0.003 ($p<0.05$), indicating a significant difference between the treatment group and the control group in the percentage of wound healing. The data were then retested using the *Mann Whitney-U* test to identify groups with significantly different relationships.

Table 9. *Mann Whitney-U* Test Results of Saba Banana Peel and Bay Leaf Extracts on Wound Healing Percentage

Treatment	P Value
K+ x K-	0.014*
F1 x K+	0.014*
F1 x K-	0.559
F1 x F2	0.059
F1 x F3	0.020*
F2 x K+	0.014*
F2 x K-	0.021*
F2 x F3	0.081
F3 x K+	0.131
F3 x K-	0.020*

Note: *significant

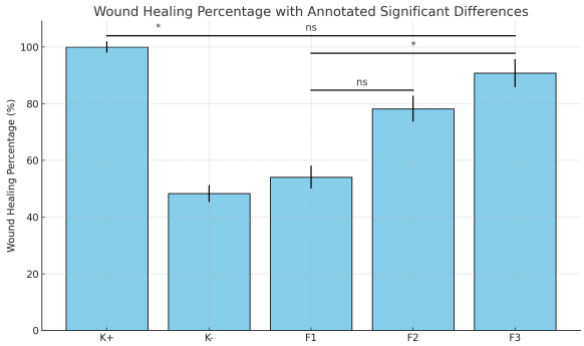

Figure 4. An Illustrating the Wound Healing Percentages With Error Bars

Measurements were made by calculating the percentage of wound healing from day 1 to day 7. Significant differences (*p<0.05) are annotated directly on the Figure 4, while non-significant differences are marked as "ns" (if applicable). The *Mann Whitney-U* test results (Table 9) show a significant difference between the K+ treatment group and the K-, F1, F2, and F3 groups (p<0.05). Based on the *Mann Whitney-U* test results, both F1, F2, and F3 show higher effectiveness compared to K+ in their effect on the percentage of wound healing. The F3 formulation (75% banana peel: 25% bay leaf) demonstrated the highest wound healing efficacy, likely due to the abundant flavonoids in banana peel extract, which modulate inflammatory mediators and enhance fibroblast proliferation.

The wound healing of white rats was observed macroscopically in each treatment group (Figure 5). The wound healing was observed for 7 days after the skin of each white rat was incised, and healing occurred within the range of 3 - 7 days (Figure 6).

The F3 formulation showed a 90.75% wound healing rate, outperforming both the positive control (*Bioplacenton*) and F1, highlighting its potential as a natural alternative for wound care. The K+ group showed an average percentage of incision wound healing of 100%, indicating a very good wound healing ability. The K- group showed an average percentage of incision wound healing of 48.3%, indicating a lower effect compared to the three gel formulations. Gel F1 had an average percentage of incision wound healing of 54.125%. Gel F2 showed an average percentage of incision wound healing of 78.225%, indicating a significant improvement compared to Gel F1. These results illustrate that the K+ group (*bioplacenton*) and the F3 treatment group (75% Saba banana peel: 25% bay leaf)

have more optimal efficacy as anti-inflammatory agents, and the higher the concentration of Saba banana peel extract, the more optimal it is in accelerating the incision wound healing process. These findings are reinforced by macroscopic observations on F1, F2, and K- which show that the wounds are still somewhat open and show signs of inflammation.

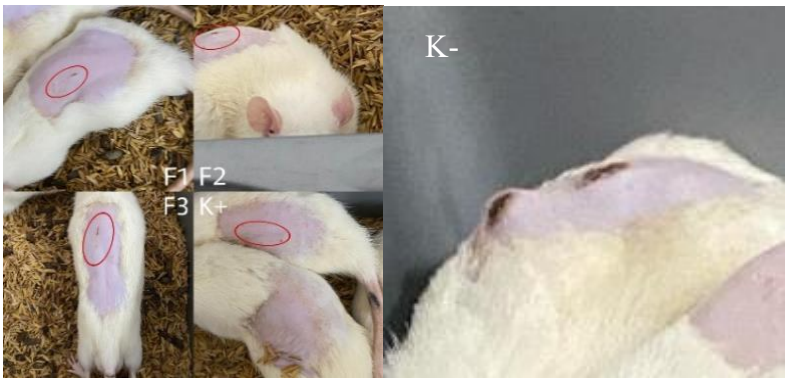

Figure 5. Anti-inflammatory Gel Activity Test Results on White Rat Test Animals

Figure 6. Anti-inflammatory Activity Before (left) and After (right) Treatment on White Rat Test Animals

The enhanced wound healing observed with F3 can be attributed to the synergistic action of bioactive compounds. Flavonoids inhibit *cyclooxygenase* (COX) and *lipoxygenase* pathways, reducing inflammation, while tannins promote collagen cross-linking, accelerating tissue repair. These findings align with studies by Agampodi et al., (2022), who demonstrated that banana peel extract enhances angiogenesis and collagen synthesis. Similarly, Kusumastuti & Jaya, (2022) reported that bay leaf extract reduces pro-inflammatory cytokines, consistent with the observed reduction in wound inflammation.

The flavonoid compounds in Saba banana peel help the performance of cells in the remodeling, inflammation, and cell proliferation phases. Flavonoids work by inhibiting *cyclooxygenase* and *lipoxygenase*, thus shortening the inflammation time [22]. The *methyl salicylate* content has anti-inflammatory properties that can help reduce inflammation in incision wounds. This is important because inflammation can slow down the healing process. *Methyl salicylate* works by inhibiting the *cyclooxygenase* (COX) enzyme, which is responsible for the production of prostaglandins that can trigger inflammation. *Methyl salicylate* can also help increase blood flow to the wound area, which can speed up healing by bringing more nutrients and *oxygen* to the injured area (Guo et al., 2022).These findings are reinforced by macroscopic observations indicating almost complete closure and drying of the wound components.

The research are in line with a previous study by Hong et al., 2023, the combination of Saba banana peel extract gel and bay leaf extract can potentially

enhance the anti-inflammatory and antibacterial activities in incision wounds. Saba banana peel extract has been shown to possess anti-inflammatory properties, inhibiting interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) secretion. On the other hand, bay leaf extract has demonstrated wound healing and anti-inflammatory effects, promoting tissue regeneration and reducing inflammation [24]. The synergistic effect of these two extracts may lead to improved wound healing outcomes by accelerating the healing process, reducing inflammation, and inhibiting bacterial growth. Additionally, the presence of bioactive compounds like oleic acid, linoleic acid, and oleamide in Saba banana peel extract [20] and compounds like ipolamiide, verbascoside, and iso-verbascoside in bay leaf extract [21] can provide an anti-inflammatory effect, with the most effective doses of 70 percent and 80 percent ethanol extract concentrations at 150 mg/kg BW, and the aqueous extract greetings at 50 mg/kg BW. The use of a solvent mixture of alcohol and water is an ideal solvent because it is the best extraction solvent for almost all low molecular weight compounds such as saponins and flavonoids.

The F3 gel formulation offers a promising alternative to conventional treatments like povidone-iodine, which, despite its efficacy, has been associated with delayed fibroblast activity and potential cytotoxicity. This study did not assess the histopathological changes in wound tissues, limiting the understanding of cellular mechanisms underlying the observed effects. Future studies should include histological analyses to evaluate collagen deposition and inflammatory cell infiltration.

CONCLUSION

The combination of Saba banana peel and bay leaf extracts in gel formulations exhibits significant antibacterial and anti-inflammatory activities, with F3 (75% banana peel: 25% bay leaf) showing the highest wound healing efficacy. These findings highlight the potential of natural extract-based gels as cost-effective, low-toxicity alternatives to conventional wound care treatments. Further research is recommended to validate these results through histopathological studies and clinical trials.

Further research should be conducted to compare the activity of this ointment with existing conventional drugs, as well as to perform toxicity tests and clinical trials in humans to evaluate its safety and effectiveness before it can be widely applied in clinical practice. Further research on the histopathological changes induced by this combination could provide a deeper understanding of its effects on collagen, neutrophil cells, monocytes, and lymphocytes in the wound healing process.

AUTHORS' CONTRIBUTIONS

Nur An-nuha Muniroh: Conceptualization, Writing – Original Draft, Writing – Review & Editing**; Winda R Cahyaningrum:** Investigation, Formal Analysis, Project Administration**; Deliza Dhiakhalda:** Methodology**,** Formal Analysis, Software**; Respatiningtyas A. D. Putranti:** Resources, Investigation**; Noha Hanifa:** Investigation, Visualization**; Kurnia R. Dhanti:** Validation, Supervision**; Dita P. K. Wardani:** Data Curation, Supervision**; Arif Mulyanto:** Data Curation, Supervision

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DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT

The authors have no competing interests to declare.

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