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Effectiveness of a Combination Gel of Bilimbi Leaf and Aloe Vera Extract for Healing Burns in Male White Rats

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ABSTRACT

Burns are a global public health problem, with approximately 180,000 deaths each year. The incidence of second-degree burns in Indonesia accounts for 46.7%. Redness, swelling, ulcers, and severe pain characterize these wounds. This study aims to test the effectiveness, percentage of healing, and length of gel application of a combination of star fruit leaf extract and aloe vera leaf pulp on the healing of second-degree burns in male rats. Morton's method was used in this study to measure the diameter of the burn wound. The treatments of this study included a combination gel of star fruit leaf extract and aloe vera leaf with variations in the ratio of F1 (6%: 5%), F2 (6%: 10%), F3 (12%: 5%), and the control group with 5 replicates each group. The results showed that the gel combination of extracts of star fruit leaves and aloe vera leaves effectively treated burn wounds in rats. Duncan's test of the treatment of variations in extract concentration showed a significant difference. The combination of starfruit leaf extract and aloe vera in gel preparation has effectively healed burns in male mice. The duration of administration is 16 days, with the percentage of healing for categories F1, F2, and F3 being 45%, 55%, and 61%, respectively. Burn wound healing activity occurs because the two extracts used contain alkaloids, flavonoids, saponins, and tannins, which act as anti-inflammatories, increase the growth of epithelial cells and collagen, and help the formation of new tissues.

INTRODUCTION

Anyone can experience burns and can occur in various places, caused by radiation, fire, electricity, hot water, chemicals, and cold trauma (Ministry of Health Republic Indonesia, 2019). The World Health Organization (WHO) states that burns are a global public health problem that results in approximately 180,000 deaths each year. WHO also states that burns are prevalent in low-income countries, one of which is Southeast Asia, and women are at higher risk of experiencing them due to household activities (World Health Organization, 2023). Burns have a relatively large number of cases (high volume) and have a high risk of morbidity and mortality (high risk), so it tends to require high costs and resources (Kemenkes, 2019). Redness, swelling, ulcers, and severe pain characterize these wounds. The incidence of second-degree burns in Indonesia reaches 46.7% of all burn incidents, which often occurs in children and adult women (Afiani et al., 2019).

Bilimbi plants are known to have the potential to help the wound healing process. Usually, the part of the plant that is widely used is the leaves, which contain secondary metabolites including alkaloids, flavonoids, saponins, and tannins (Sari et al., 2023). Pendit's research (2016) states that bilimbi leaves have anti-inflammatory properties derived from secondary metabolites of tannins, saponins, and flavonoids. The concentration of bilimbi leaf extract in gel preparations of 6%, 9%, and 12% gave the most optimal burn wound healing results at a concentration of 12% (Putri, 2022).

Another herbal plant that is efficacious as a burn healer is aloe vera (Aloe vera). This plant is familiar to the public because it is known (Khan et al., 2013). Aloe vera is a plant that contains

secondary metabolite compounds such as anthraquinones, saponins, flavonoids, and tannins (Sari & Raharjo, 2019). The roots of this plant have secondary metabolites of flavonoids and saponins, while the flesh of the leaves contains alkaloids, flavonoids, saponins, and tannins (Kulsum, 2020). Saponins are used as a cleanser to help heal open wounds, while tannins help prevent infection because of their antiseptic power. Flavonoids can act as an antiseptic, which is proven in antibacterial testing against the growth of Staphylococcus aureus bacteria (Pratama, 2018). Anggraini's research (2019) shows that aloe vera leaf extract at 10% in gel preparations has provided burn wound healing activity in rats.

Morton's method allows standardization in the preparation of burns, both in terms of depth and area of the wound. This method can obtain a more accurate measurement of the burn response and its healing, thus helping to evaluate the effectiveness of therapies or drugs being tested. Research by Rokhmah et al. (2021) and Indriani (2020) on burn wound healing also used the Morton method. The study's novelty is that the combination gel of bilimbi leaf extract (Averrhoa bilimbi L.) and aloe vera leaves is highly effective in healing burn wounds in male white rats. The update is done by reducing the concentration of extracts that are known to be effective. This is expected to provide synergistic effectiveness results from both extracts to optimize the burn wound healing process.

METHOD

This study has received ethical clearance from the Animal Use Ethics Committee, Universitas Pakuan, with the number 022/KEPHP-UNPAK/05-2024.

Preparation of bilimbi leaf and aloe vera leaf

Fresh bilimbi leaves, as much as 4 kg, were wet sorted, washed with running water until clean, and drained. The leaves were dried using an oven for 24 hours at 40 °C until dry. Then dry sorting was carried out. Next, the sample was pulverized using a grinder until it became simplisia powder, then sieved using a 40-mesh sieve until a fine powder was obtained. Weighed the simplified powder obtained, and stored it in a clean jar, tightly closed, and protected from sunlight (Andriani, 2019).

Fresh aloe vera leaves totaling 17 kg were wet sorted, washed under running water until clean, and drained. The skin of aloe vera leaves and the thorns is peeled using a knife. The flesh of aloe vera leaves is freeze-dried until dry, then mashed using a grinder and sieved with a 40-mesh sieve. Weighed the simplified powder obtained and stored in a clean jar, tightly closed, and protected from sunlight (Yusuf et al., 2019; Wahyuningsih et al., 2021).

Preparation of bilimbi leaf extract and aloe vera leaf extract

Extraction of this wuluh bilimbi leaf uses the maceration method. The ratio of materials and solvents used is 1:10. Bilimbi leaf simplisia powder was put into a glass jar, then 70% ethanol was added, stirred, and closed. Then the glass jar is covered with aluminum foil. Let stand in a closed place for 3x24 hours and stir every 1x24 hours for 5 minutes. The residue and filtrate were poured and filtered using filter paper. The filtrate obtained is concentrated using a rotary evaporator until a thick bilimbi leaf extract is obtained (Hasim et al, 2019).

Dry powder of aloe vera leaf simplisia was put into a container, then extracted by the maceration method using 70% ethanol, and allowed to stand for 3 × 24 hours. Stirring was carried out every 1x24 hours for 5 minutes. After 72 hours, it was poured and filtered using filter paper, and the filtrate was collected. The filtrate is collected and then evaporated with a rotary evaporator at 40- 45°C until aloe vera extract is obtained (Wahyuningsih et al., 2021).

Characteristic testing of bilimbi leaf and aloe vera extracts determination of moisture content

The determination of moisture content was carried out using the azeotrop distillation method.

1. Toluene Saturation
   200 mL of toluene and 2 mL of distilled water were put into a round-bottom flask, installed with a distillation device, and distilled for 2 hours until the water droplets were gone. Distillation was stopped and allowed to cool for 30 minutes, and the volume of water in the receiver tube was read with an accuracy of 0.05 mL.
2. Determination of Extracted Water Content
   5 grams of extract were put into a flask containing saturated toluene, then heated for 15 minutes. After boiling toluene, distillation was set at a speed of 2 drops/second until some of the water was distilled, then the distillation speed was increased to 4 drops/second. After all the water was distilled, the inside of the cooler was rinsed using toluene. Then, continued distillation for 5 minutes, and then the receiving tube was cooled at room temperature. After the water and toluene were completely separated, the volume of water was read. The difference between the two volumes of water is read according to the water content contained in the material being examined (Utami, dkk., 2017).

Determination of ash content

The sample, which weighed as much as 2 grams, was put into a silicate crucible that had been incinerated and tared and slowly incinerated in a furnace at 600 °c until it became ash. The ash obtained was cooled and weighed (Evifania et al., 2020).

Phytochemical screening test

Phytochemical tests were conducted on the saponins and extracts, including alkaloids, flavonoids, tannins, and saponins. The sample was reacted with Mayer, Bouchardat, and Dragendroff reagents for the alkaloid test. In the Flavonoid test, the sample was reacted with Mg powder and concentrated HCl. In the tannin test, the sample was reacted with FeCl3. In the Saponin test, the sample was reacted with 2N HCl (Artini et al., 2014). Flavonoid compounds act as an anti-inflammatory agent and antioxidant, preventing blockage of blood vessels (Wardani, 2018).

Gel preparation and evaluation

Carbopol was dissolved in hot water until a mucilago gel base was formed (mass 1). After that, benzoic acid, glycerin, and propylenglycol were dissolved into water in a beaker glass (mass 2). The extracts of bilimbi leaf and aloe vera were dissolved into water in another beaker glass (mass 3). Mass 3 was added to mass 1 and 2 and stirred with a homogenizer until a homogeneous mass was obtained. The last step, distilled water is added until the mass of the gel becomes 100 grams (Wahyuningsih et al., 2021). The preparation that has been made is continued with organoleptic, homogeneity, pH, viscosity, and spreadability testing (Erwiyani et al, 2020).

Treatment of experimental animals

The preparation and grouping of experimental animals begins with an ethical review of experimental animals at the Pakuan University Animal Ethics Committee to ensure that the procedures to be carried out meet animal ethical review. In this study, the samples used were 25 Sprague-Dawley male white rats aged 2 to 3 months and weights ranging from 170 to 200 grams. The rats used were acclimatized for 1 week. During the process, the rats were given a drink and fed ad libitum daily. Before and after completion of acclimatization, rats were weighed, and the CV (Coefficient of Variation) was calculated to determine the uniformity of the rats used. Burns

were made based on the Morton method by injecting rats intraperitoneally using a combination of Ketamine 0.05 mg/gBB and Xylazine 0.005 mg/gBB.

Furthermore, the rat's back was shaved ± 3 cm in size and cleaned, targeting the wound area with 70% alcohol disinfectant. Burns are made with an iron plate with a diameter of 1.5 cm which is previously heated using fire for 3 minutes, then attached to the left back of the rat for 7 seconds until a second degree burn is formed which is indicated by redness and the formation of water bubbles (bula) on the rat's skin (Tumigolung, 2019; Indriani, et al., 2020). The treatment process was carried out in each treatment group, starting 5 minutes after the wound was created (Wijayantini, 2018). Treatment was carried out by applying 200 mg of gel preparation to the wounded part of the rat's back using a thin and evenly distributed stirring rod. Gel preparations are carried out twice a day, in the morning and evening, until the burn heals (Indriani et al., 2020). The 25 rats were randomly grouped into five groups with 5 replicates of each treatment. The treatment groups of this study include positive control (Bioplacenton gel), negative control (gel base), F1 (6% bilimbi leaf extract and 5% aloe vera), F2 (6% bilimbi leaf extract and 10% aloe vera), and F3 (12% bilimbi leaf extract and 5% aloe vera).

Table 1. **Gel of combination gel of bilimbi leaf and aloe vera extract**

Material	Concentration (%w/w)				Function
	K (-)	F1	F2	F3	
BL extract	-	6	6	12	Active Ingredient
AV extract	-	5	10	5	Active Ingredient
Carbopol	0,5	0,5	0,5	0,5	*Gelling agent*
Benzoic Acid	0,5	0,5	0,5	0,5	Preservatives
Propilenglikol	25	25	25	25	Cosolvent
Gliserin	15	15	15	15	Humectant
TEA	0,1	0,1	0,1	0,1	pH adjustment
Aquadest ad	100	100	100	100	Solvents

BL extract*: Bilimbi leaf extract
AV extract*: Aloe vera extract

Total plate count testing

The test portion is prepared by weighing 10 g into 90 mL soybean casein digest broth (added with a neutralizing agent, 0.05% polysorbate 80) and then homogenized. Dilution 10^{-2} was done by mixing 1 mL of the sample concentration 10^{-1} with 9 mL of diluent. Further dilution is done by mixing 1 mL of the previous concentration into 9 mL of diluent, until it reaches the desired concentration. At the dilution stage, the same dilution medium was used when making the initial suspension. Inoculation was done by adding 1 mL of the sample into the dish. Then liquid TSA (45 ± 1 °C) was poured, and allowed to solidify (TSA medium should be poured no more than 1 hour after inoculation). The work was done in duplicate. The dishes were inverted and incubated at 30-35°C for 3-5 days.

The results were interpreted by selecting the dishes that showed colony counts <250. Total aerobic microbial count (TAMC) was equivalent to the CFU value found using TSA media. Results were expressed as CFU per gram (<10 colonies per gram when no colonies were found to grow). (U.S. Pharmacopeia, 2020).

Burn diameter observation and measurement

Observation of wound diameter in this study was carried out using the Morton method, namely by measuring four wound diameters (horizontally, vertically, and two diagonally) until day 16, then calculating the average value of the diameter of each measurement using a caliper. The treatment process of burn wounds in rats was carried out routinely every day, while the measurement and data collection of wound diameter in rats was carried out every 3 days on days 1, 4, 7, 10, 13, and 16 (Putri, 2022).

RESULTS

Table 2. Extract characteristics

Sample	Moisture content	Req	Conclusion	Ash content	Req	Conclusion
BL extract	8,4%	<10%	Qualified req	3,65%	<7,5%	Qualified req
AV extract	9,35%			6,7%		

Moisture content indicates the amount of water present in the extract, which is very important for determining its stability and shelf life. The test results showed that both extracts had the appropriate water content and could be stored properly without risk of damage or spoilage.

Ash content describes the amount of minerals in the extract after combustion. A high ash content can indicate the presence of certain essential minerals, but if it is too high, it can reduce the quality of the extract due to excessive mineral content. Based on the test results, both extracts showed good quality and can be used for further research by established standards in plant extract processing.

Table 3. Phytochemical test

Test	Bilimbi leaf extract	Aloe vera extract
Alkaloids (Dragendroff)	+	+
Alkaloids (Mayer)	+	+
Alkaloids (Bourchardat)	+	+
Flavonoids	+	+
Saponins	+	+
Tannins	+	+

The table shows the phytochemical test results for star fruit leaf extract (Bilimbi leaf) and aloe vera extract (Aloe vera). Both extracts showed the presence of alkaloids (in Dragendorff, Mayer, and Bourchardt tests), flavonoids, saponins, and tannins. This suggests that both extracts contain bioactive compounds that have potential as anti-inflammatory, anti-microbial, and wound-healing agents, which may contribute to the therapeutic effects of both plants.

Table 4. Gel evaluations

Evaluations	Parameters	F1	F2	F3	K(-)
Organoleptic	Aroma	-	Typical aromatic	Typical aromatic	Strong Typical aromatic	Unscented
	Texture	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid
	Color	-	Blackish green	Blackish green	Blackish green	Clear colorless
Homogeneity		Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH		4,5-6,5	5,79	5,13	5,06	6,06
Viscosity		2.000-5.000 cP	8.140	9.875	9.920	6.540
Spreadability		5-7 cm	5,91	5,54	5,30	6,23

The table presents gel evaluation results for formulations F1, F2, F3, and the control, focusing on organoleptic properties, texture, pH, viscosity, and spreadability. Aroma, texture, and colour are crucial for user acceptability, with F1 having no aroma, F2 and F3 exhibiting typical aromas, and the control being unscented. All gels have a semi-solid texture, which is essential for stability and easy application. The colour of F1, F2, and F3 is blackish-green, likely due to plant extracts, while the control is precise. Homogeneity ensures the consistent distribution of active ingredients. pH is important for skin compatibility, with slightly acidic formulations preferred for better absorption and less irritation. The viscosity of F1, F2, and F3 is higher, suggesting thicker gels with more active ingredients. Spreadability is slightly higher in the control, but all formulations show adequate application properties. These results highlight the importance of balancing texture, pH, viscosity, and spreadability for effective and comfortable gel formulations.

Table 5. Total plate count testing

Sample	Colony count	Requirements
K(-)	<10	$<10^3$ CFU/gram(BPOM, 2019)
F1	<10	
F2	<10	
F3	<10	

The table shows the total plate count testing results for samples K(-), F1, F2, and F3, all with a colony count of less than 10 CFU/gram. This is well below the required standard of $<10^3$ CFU/gram, as BPOM (2019) specified, indicating that the samples are within acceptable microbial limits.

Table 6. Average diameter of burn wounds in rats

S	Burn diameter Day- (cm)							
	0	1	4	7	10	13	16	x
F1	1,50^{h}±0	2,38^{p} ± 0,068	2,096^{mn} ± 0,057	1,798^{k} ± 0,064	1,72^{jk} ± 0,064	1,312^{g} ± 0,049	0,764^{de} ± 0,055	1,653 ±0,529
F2	1,50^{h}±0	2,248^{o} ± 0,03	1,95^{l} ± 0,044	1,62^{i} ± 0,055	1,108^{f} ± 0,046	0,812^{e} ± 0,056	0,432^{c} ± 0,131	1,381 ±0,638
F3	1,50^{h}±0	2,01^{lm} ± 0,075	1,674^{ij} ± 0,061	1,34^{g} ± 0,034	0,772^{de} ± 0,055	0,334^{b} ± 0,058	0,082^{a} ± 0,113	1,102 ±0,719
K+	1,50^{h}±0	2,01^{lm} ± 0,059	1,674^{ij} ± 0,056	1,336^{g} ± 0,031	0,718^{de} ± 0,117	0,282^{b} ± 0,171	0,044^{a} ± 0,099	1,081 ±0,742
K-	1,50^{h}±0	2,3^{op} ± 0,036	2,1^{1n} ± 0,044	1,92^{lm} ± 0,033	2,002^{lk} ± 0,033	1,946^{l} ± 0,028	1,78^{k} ± 0,039	1,945 ±0,253

The table displays rats' average burn wound diameter over a 16-day period for different groups: F1, F2, F3, K+, and K-. At day 0, all groups had similar burn wound diameters (around 1.5 cm). Over time, the burn diameters decreased in all groups, with F1, F2, and F3 gradually reducing wound size, indicating healing. By day 16, the F3 group exhibited the smallest wound diameter (0.764 cm). The K+ group had a larger wound diameter (1.081 cm), suggesting more significant healing in the experimental groups compared to the control groups (K+ and K-). The letters (a, b, c, etc.) indicate statistical differences between the groups at each time point, showing that F1, F2, and F3 had more significant reductions in wound diameter compared to the control groups. The final wound size (x) after 16 days varied, with F3 showing the best healing outcome.

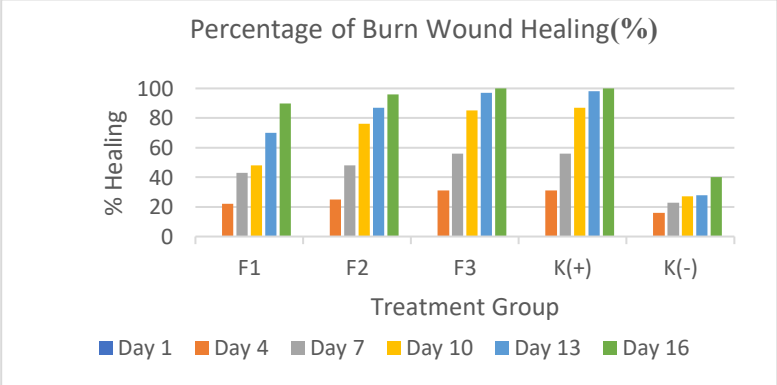

Figure 1. Percentage of burn wound healing

The chart displays the percentage of burn wound healing over 16 days for different treatment groups: F1, F2, F3, K(+), and K(-). On Day 1, all groups show minimal healing, but by Day 4, the healing percentages begin to increase. The F3 group demonstrates the most significant healing progression, especially on Day 16, with the highest percentage of healing. The K(+) and K(-) control groups show slower healing compared to the experimental groups (F1, F2, F3). By Day 16, F1, F2, and F3 exhibit higher healing percentages, with F3 leading in overall wound recovery. The healing rate for each group varies over time, but the experimental treatments (F1, F2, and F3) consistently show more significant healing compared to the control groups.

D	Treatment				
	K(+)	K(-)	F1	F2	F3
Day 1					
Day 4					
Day 7					
Day 10					
Day 13					
Day 16					

Figure 2. Visual burn observation results

The images show burn healing progress over 16 days in different treatment groups. On Day 1, all groups have similar wounds. By Day 4, the treated groups (F1, F2, F3) show better healing compared to the control groups (K(+) and K(-)). By Day 16, F1, F2, and F3 show significant healing, with the control groups lagging. F3, in particular, exhibits the most advanced healing by the end of the study.

DISCUSSION

Based on the study's results, the water content of bilimbi leaf simplisia and aloe vera extract was 9.35% and 8.4%, respectively. The results indicate that the sample's water content has met the requirements according to the MMI (Materia Medika Indonesia) reference, which is less than 10% (Departemen Kesehatan RI, 1995). Moisture content in simplisia and extracts of more than 10% or higher may cause a decrease in quality and mold growth so that the material can be damaged (Departemen Kesehatan RI, 2000).

Ash content should have a small percentage because this parameter indicates the presence of heavy metal contamination resistant to high temperatures (Maryam, et al., 2020). Based on the study's results, the ash content of bilimbi leaf extract and aloe vera leaf flesh extract was 3.65% and 6.7%, respectively. The results indicate that the sample's ash content has met the requirements according to the MMI (Materia Medika Indonesia) reference, which is not more than 7.5%.

Phytochemical test

Based on the results of the phytochemical test, it was found that the simplisia powder and thick extract from the sample of bilimbi leaf and aloe vera showed that both contained active components, including alkaloids, flavonoids, tannins, and saponins. Samples of simplisia powder and thick extracts of bilimbi leaf and aloe vera are positive for alkaloids characterized by the formation of a white precipitate when reacted with Mayer reagent, brick red color when reacted

with Dragendorff reagent, and brown precipitate when reacted with Bouchardat reagent; the resulting precipitate is a potassium-alkaloid complex. Both samples are also positive for flavonoids, indicated by the formation of an orange color. This results from the coordination covalent bond between magnesium ions and the phenolic OH groups of flavonoid compounds. The formation of a blackish green color indicates the presence of tannins because when FeCl3 is added, the sample will react with Fe3+ ions and will form a trisianoferritic potassium Ferri(III) complex compound (Halimu, et al., 2017). In addition, the formation of a constant froth indicates that the sample contains saponins because when shaking, the hydrophobic group binds to air. In contrast, the hydrophilic group binds to water to form a froth (Suleman, et al., 2022). The results are by previous research (Hasim et al., 2019; Sari & Raharjo, 2019) that bilimbi leaf and aloe vera showed that both contained active components, including alkaloids, flavonoids, tannins, and saponins.

Gel evaluations

Evaluation of gel preparations included organoleptic, homogeneity, pH, viscosity, and spreadability. The results of the gel preparation evaluation can be seen in Table 4. The organoleptic test is intended to determine the physical preparation of the gel made by looking directly. Based on the results of the study, it was found that the negative control, which is the base of the gel, is odorless and colorless due to the absence of the addition of extracts intended as a negative control or preparation that does not have any effect on the healing of burns in rats. Formula 3 has a more intense color and a stronger aroma than formulas 1 and 2 because the amount of concentration of active substances used is the largest, this shows that the higher the concentration of extracts in a formula, it will have an effect even though the intensity of the difference is not too significant with other formulas.

The homogeneity test aims to ensure that the gel preparation has no lumps. This test found that all preparations showed homogeneity supported by an even color, no lumps, and coarse particles on the object glass, so it can be concluded that there were no differences in homogeneity in the four preparations. This indicates that the preparation made is in accordance with the requirements of SNI No. 06-2588, namely gel preparations must be evenly mixed and there must be no coarse lumps.

Testing the pH of the gel preparation aims to determine whether or not the gel preparation is safe for the skin to accept. Preparations with too low a pH can irritate the skin, while preparations with too high a pH can cause the skin to dry out. The results showed that the gel preparation had a pH between 5.06 and 6.06. The data show that the higher the amount of extract used, the lower the pH of the gel preparation obtained (Rosida et al., 2018). The pH value of gel preparations in this study shows that all dosage formulas are safe for skin acceptance because they are in the range of 4.5-6.5 according to the requirements of SNI No. 06-2588-1992.

Measurement of the gel preparation's viscosity aims to determine the gel preparation's viscosity, which describes the amount of resistance of a liquid to flow. The results showed that the gel preparation produced had a viscosity between 6,540-9,920 cP. This shows that the preparation is by the SNI No. 16-4380-1996 standard; the viscosity value of the gel preparation is in the range of 2,000-50,000 cP. The data obtained shows that the higher the concentration of the extract, the higher the viscosity value; the viscous nature of the extract can cause this.

The measurement of spreadability aims to determine the potential distribution of gel preparations in the area of use. Gel preparations can spread nicely and evenly if they have a low viscosity. The results showed that the resulting gel preparation had a spreadability ranging from 5.30-6.23 cm, which shows compliance with the standards set by SNI No. 06-2588 that a good gel preparation has a spreadability of 5-7 cm. Based on the data obtained, the higher the concentration of extract used, the smaller the spreadability produced. This is due to the higher the concentration of the extract, the higher the viscosity of the preparation, which causes a small spreadability. The greater the spreadability of the preparation, the more the active substance can spread and contact the skin more widely. Preparations exhibiting homogeneity are conducive to achieving optimal outcomes due to their uniform composition of active principles (Tari & Indriani, 2023).

Total plate count testing results

The total plate count analysis results for gel preparations of bilimbi leaf and aloe vera extracts showed that the TPC value in the samples replicated twice was below the detection limit (<10 colonies/gram). The results indicate that the gel preparation has an excellent level of microbiological hygiene, because the number of microorganisms detected is very low, which is less than 10 colonies per gram. According to the Food and Drug Administration Regulation Number 12 of 2019, this still meets the requirements of less than 103 CFU/gram (BPOM, 2019). These results meet the requirements and can be caused by extracts of bilimbi leaf extract and aloe vera leaves, which have antibacterial properties (Wijayanti & Safitri, 2018; Sofia et al., 2023). Bilimbi leaf extract has ingredients that act as antibacterials, namely flavonoids, terpenoids, and tannins (Astuti et al., 2016), while aloe vera leaf extract has ingredients that act as antibacterials, namely alkaloids, flavonoids, and tannins.

Observation and measurement of burns in rats

Burn wound healing in rats in this study was pursued by applying the gel twice daily at 08.00 am and 02.00 pm. Observation and measurement of burn wounds in rats were carried out every 3 days (days 1, 4, 7, 10, 13, and 16) for 16 days. This is because, these days, there is an increase in wound healing (Dwivedi et al., 2016). Burn wound diameter was measured using the Morton method, which measures 4 diameters from 4 different sides for 16 days using a caliper (Wijayantini, 2018). Data on the average diameter of rat burns can be seen in Table 6.

The results of measuring the diameter of burns on rats obtained were then subjected to statistical tests using factorial Completely Randomized Design analysis with the SPSS program. Based on statistical tests, it was found that there was a very significant effect of treatment, day, and interaction between treatment and day on the diameter of burn wounds in rats because the significance value of 0.000 was obtained, which indicated that it was smaller than the real level value of 0.05. This indicates rejecting H0 and accepting H1, meaning that there is a significant difference in the reduction of wound diameter in the treatment, day, and interaction between treatment and day.

The data analysis was continued with Duncan's further test to see any differences in the effect on the treatment groups. Based on this test, the treatment factor gave positive control results, and formula 3 showed the same effect on reducing the diameter of burn wounds. A decrease in the diameter of the burn wound from the treatment indicates healing. The factor of the day of data collection gave the result that all treatments had a different effect on reducing the diameter of the burn wound. Duncan's further test showed a complex interaction between treatment and day on burn diameter, with 16 significantly different subsets ($p < 0.05$). The smallest wound diameter was found in formula 3 on day 16, so its healing ability can be similar to the positive control or Bioplacenton gel already on the market, indicated by both being in the same subset. Formula 2 showed a larger wound size than Formula 3, while Formula 1 had a larger wound size than Formula 2 and Formula 3. This is indicated by the large diameter of the wound of formula 1 on day 16 being in the same subset as formula 3 on day 10. All treatments showed a significant decrease in wound diameter over time, except for the negative control, which consistently had the largest wound diameter at each time point because no active substances in the gel preparation could provide therapeutic effects.

Percentage of burn wound healing

The results of the percentage of burn wound healing showed variations in effectiveness between treatments for 16 days of observation. Based on the data obtained, there was an increase in the wound healing process in all treatments, which was characterized by an increase in the percentage of burn wound healing. Formula 3 and the positive control showed the best performance, achieving perfect skin condition (100%) on day 16, followed by Formula 2 (96%) and Formula 1 (90%). The negative control showed the slowest healing, only reaching 40% on day 16. In the negative control group, there was still a decrease in the size of the wound diameter

even though there was no active substance contained in the preparation, this was due to the natural healing process of the rat's body itself but in a longer period compared to the use of active substances such as those contained in positive controls, F1, F2, and F3. The healing pattern was progressive for all treatments except the negative control, with Formula 3 and the positive control showing the fastest healing rate, followed by Formula 2, then Formula 1, indicating the different effectiveness of each formula in accelerating the burn wound healing process.

Visual burn observation results

The results of visual observation of burns are also related to the results of measuring the diameter and percentage of wound healing, namely the diameter of the wound in the positive control, formulas 1, 2, and 3 has decreased, causing an increase in the percentage of healing as well as the visual condition of burns in rats which shows that the combination gel of star fruit leaf extract and aloe vera leaf pulp has effectiveness with cell regeneration, antibacterial, and anti-inflammatory against burn wound healing just like Bioplacenton gel. All formulas showed effectiveness in healing burns, but only F3 had the most similar results to the positive control. The negative control had no wound-healing effect because the gel base preparation contained no active substances to help the burn healing process.

Burn wound healing is divided into 3 phases: inflammatory, proliferative, and remodeling. In the inflammatory phase, there is swelling and blackness. In the proliferation phase, exudate and fibroblasts or scabs form at the top of the wound. In the remodeling or healing phase, the formation of new tissue indicates that the wound has shrunk or healed (Izzati, 2015). Rats in all groups experience these three phases at varying times. The results of burn wound healing can be seen in Figure 2.

On the first day of observation or 24 hours after wound formation, all groups showed blackish, red, wet, and swollen wounds on the rat's back (visual characteristic 1). This indicates the inflammatory phase, which can occur up to 3 days after wound formation. The wound healing process will not occur without the inflammatory phase. This phase controls bleeding, prevents bacteria from entering, removes debris from the wound tissue, and prepares the wound for further healing. The wound will remain painful from the inflammatory phase until healing (Izzati, 2015).

On the fourth day after wound formation, blackish scab formation and swelling occurred in the positive control group, formulas 1, 2, and 3 (visual characteristic 2). Meanwhile, the negative control group had scab formation on day 10. This phase is called proliferation, the start of the wound healing process, characterized by exudate and fibroblasts that look dry above the wound (Izzati, 2015). The proliferation phase is assisted by fibroblasts, which are cells that produce collagen. Then collagen works by connecting the tissue in the burn wound to restore skin tissue and accelerate burn wound healing (Priamsari & Yuniawati, 2019). On day 7, the positive control group, formulas 2 and 3, showed that the scab began detaching from the skin and forming reddish-colored tissue (visual characteristic 3). This indicates that these groups have reached the end of the proliferation phase, which means the rats of these treatment groups will continue the maturation phase. Meanwhile, the Formula 1 group on day 10 showed that the scabs were still not detached, and the negative group had just formed scabs.

The final phase of wound healing is remodeling or maturation. During this phase, the new tissue formed will be arranged to resemble the original tissue (Priamsari & Yuniawati, 2019). This final phase began on day 7 for positive control 1, formula 2, and 3, while formula 1 was on day 13 and negative control on day 16. The positive control group showed reddish skin and drying on day 10 (visual characteristic 5), while the formula 2 and 3 groups were still reddish and shriveled slightly (visual characteristic 4). On day 13, the positive control showed that the skin had returned to the beginning (visual characteristic 6). However, the formula 2 and 3 groups were still reddish and dry (visual characteristic 5), formula 1 showed a detached scab which means it reached the peak of the proliferation phase (visual characteristic 3).

In contrast, the negative control group still formed a scab (visual characteristic 2). On day 16, the positive control group and formula 3 showed pale skin condition and no marks (visual characteristic 6) indicating that healing had occurred. The skin had returned to its initial state,

formula groups 1 and 2 showed wounds were still reddish (visual characteristic 5). The negative control had only scabs detached which means it has reached the peak of the proliferation phase (visual characteristic 3). This result shows that formula 3 is most similar to the positive control in healing burn wounds. The treatment of formulas 1, 2, and 3 showed wound healing activity, but formula 3 was the most efficient among the other formulas in healing 2nd degree burns. The slow healing process in the negative control is due to the absence of active substances from star fruit leaf extract and aloe vera leaf pulp in the gel preparation given; this serves as a reference that formula groups 1, 2, and 3 should have results that are inversely proportional to the negative control. The burn wound healing involves tissue regeneration and re-formation of the epidermis and dermis. The healing time of rat skin after experiencing 2nd degree burns varies, but generally takes about 2 to 3 weeks to heal completely (Hasanah, 2023). This shows that the results of the study are appropriate, namely, rats recovering from second-degree burns within 2 to 3 weeks.

None of the treatment groups experienced infection during the burn wound healing observation. The signs of infection include the emergence of pus from the wound, changes in wound color, the wound feels warmer than the surrounding area, and pain that increases over time. This does not happen because the extracts used contain saponins, which function as antibacterials, so that they can prevent infection.

The high burn wound healing ability of bioplacenton gel is due to the content of 0.5% neomycin sulfate and 10% bovine placenta extract. Bovine placenta extract functions to accelerate the size reduction in wounds or neoangiogenesis through decreasing the transformation of growth factors, increasing epithelialization or the regeneration process of the epithelial layer on the wound surface to cover wounds, increasing endothelial tissue growth factors, and collagen synthesis in blood vessels and CD31+ (Sukmawan et al., 2021). Meanwhile, neomycin functions to prevent or treat gram-negative bacterial infections in the wound area (Harlis et al., 2023).

Based on the results obtained in this study, star fruit leaf extract gel and aloe vera leaf flesh have effectiveness in healing burns on male white rats, which shows consistency with previous research (Putri, 2022, & Wahyuningsih, 2021). The effectiveness of this healing is supported by the content of secondary metabolites contained in both extracts, including alkaloids, flavonoids, saponins, and tannins. Alkaloids can help the wound healing process with the mechanism of action of early dermal and epidermal regeneration, positively affecting cellular proliferation, granular tissue formation, and epithelialization (Safani et al., 2019). Flavonoids have the potential as anti-inflammatory so that they can reduce the pain of wounds experienced by mice, with the mechanism of action, namely increasing the proliferation of epithelial cells and collagen, so that the development of wound healing becomes more effective (Fahrezi & Sumarmin, 2021). Saponins can improve wound healing with the mechanism of action of triggering the formation of type I collagen in the presence of proteins that play an important role in the wound closure process and increase tissue epithelialization, and as anti-microbials to minimize the risk of infection (Putri et al., 2023). Tannins have the potential as an astringent that can stop bleeding, accelerate wound healing and inflammation, and the formation of new tissues (Mustiqawati et al., 2023). Belimbing wuluh leaf extract is known for its antibacterial ability derived from saponins, while aloe vera leaf pulp extract is known for its anti-inflammatory ability derived from flavonoids. Both potentials support the effectiveness of synergistic preparation formulations in healing burn wounds in this study. This is similar to Bioplacenton, which is used as a positive control, which has anti-inflammatory and antibacterial activities derived from bovine placenta extract and neomycin sulfate.

CONCLUSION

The combination of starfruit leaf extract and aloe vera in gel preparation has effectiveness in healing burns in male white mice. The duration of administration of the preparation is 16 days, with the percentages of healing F1, F2, and F3, respectively, of 45%, 55%, and 61%.

It is necessary to conduct a stability test on the formulation of a gel preparation of a combination of bilimbi leaf and aloe vera extract, and testing of skin irritation and sensitization of a gel preparation of a combination of bilimbi leaf and aloe vera extract is carried out.

AUTHOR'S DECLARATION

Authors' contributions and responsibilities

SN: Writing original draft, visualization, funding acquisition, conceptualization; **MTRH**: Writing original draft (supporting), funding acquisition; **DFH**: Supervision (lead), Validation (equal), Visualization (equal), Funding Acquisition (equal), Review and Editing.

Availability of data and materials

All data are available from the authors.

Competing interests

The authors declare no competing interests.

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