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Efficacy of Starfruit Leaf Ethanol Extract Gel on Second-Degree Burns in Wistar Rats

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ABSTRACT

Burn injuries are injures caused by heat, electricity, chemicals, or radiation exposure. Secondary metabolites found in starfruit (Averrhoa carambola) leaves—such as saponins, alkaloids, and flavonoids—have the potential to accelerate the healing of burn wounds. The main objective of this research is to evaluate the effects of a topical gel made from ethanol extract of starfruit leaves at concentrations of 5%, 10%, and 15%, and to determine the most effective concentration for burn wound healing. This study used an experimental method involving 25 Wistar rats, divided into 5 groups. The burn wound model was created by inducing second-degree burns on the rats' backs using a metal disc with a diameter of 2 cm, applied to the skin for 5 seconds. After the burn was induced, the gel was applied topically twice a day for 15 consecutive days. The healing process was observed macroscopically by measuring the percentage of wound closure and using a modified Nagaoka scoring system. The data obtained from this study showed that the ethanol extract gel of starfruit leaves effectively accelerated the healing of second-degree burns. Tukey's test results indicated that the 15% concentration of the gel produced a statistically significant effect compared to the negative control group ($p = 0.012$), and was identified as the most effective treatment group, with a burn wound healing percentage of 86.15±0.90%, which was noticeably higher than that of the other groups. Based on analysis using wound healing percentage and Nagaoka scores, the optimal concentration was 15% with healing time equivalent to the positive control group.

Keywords: *Burn Injury, Starfruit (Averrhoa Carambola), Gel, White Rats*.

INTRODUCTION

Burns are a complex form of injury that not only impacts the patient locally but also causes serious systemic effects through a severe and prolonged inflammatory response. Burns are caused by heat, electricity, chemicals, and radiation. The size and depth of the wound, along with location, age, and underlying medical conditions, can influence the severity of a burn. (Burgess et al., 2022). The goal of burn treatment is to restore the optimal form and function of skin tissue and reduce the side effects that arise. In society, herbal medicine is used as an alternative therapy (Vania et al., 2018). Herbal medicine has a strategic role in addressing various diseases because it has high therapeutic potential, making it widely accepted by patients with various health conditions. In herbal medicine, parts of plants, whole plants, or selective isolation of phytoconstituents are utilized as herbal medicinal materials. (Barkat et al., 2021). Therapy with herbal medicine is preferred by society because it not only has potential side effects at mild levels but also it is widely available.

One component in plants that is believed to be efficacious for burn wound healing is starfruit leaves (*Averrhoa carambola*). Based on phytochemical research conducted by Jannah (2023), starfruit leaves contain secondary metabolite compounds such as alkaloids, tannins, flavonoids, and saponins. Alkaloids have antibacterial activity that can accelerate wound healing (Trinh et al., 2022). Tannins function as antiseptics that prevent infection of burn wounds (Ola, 2020). Flavonoids act as anti-inflammatories in wound healing. (Agus Sunadi Putra et al., 2023). Saponins function as antibacterial agents that have mechanisms to reduce the membrane permeability of bacterial cells (Endriyatno et al., 2023).

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First aid for burns involves stopping the burning sensation and cooling the injured area. This cooling is effective if given within three hours of the injury. (Ramba et al., 2023). This research uses ethanol extract of star fruit leaves formulated in gel preparation. The gel which comes from its high water content provides a cooling effect when applied to the skin. (Januarti, 2024).

MATERIALS AND METHODS

Equipment and Materials

Equipment

The instruments used in this research were a blender, maceration vessel, Buchner funnel, compressor, rotary evaporator (Stuart), water bath (Memmert), analytical balance (Ohaus), pH meter (Hanna), viscometer (Brookfield), spreadability testing apparatus, adhesion testing apparatus, animal test cage, object glass, porcelain dish, beaker glass (Pyrex) 50 mL and 10 mL, test tubes, stirring rod, horn spoon, and porcelain dish.

Materials

The materials used in the research were dried simplicia of sweet starfruit leaves (*Averrhoa carambola*), Bioplacenton®, technical 96% ethanol, CMC Na, carbopol 940, technical propylene glycol, pro analysis glycerin, pro analysis Triethanolamine (TEA), pro analysis methyl paraben, distilled water, pro analysis hydrochloric acid (HCl) 2N and concentrated, pro analysis Mayer reagent solution, pro analysis Bouchardat, pro analysis Dragendorf, pro analysis Ferric Chloride ($FeCl_3$), pro analysis Magnesium powder, and white Wistar strain rats.

Methods

Research ethics

Research ethical requirements, including ethical clearance, has been assessed and approved by the Health Research Committee of RSUD Dr. Moewardi, Surakarta, on September 10, 2024, and obtained Ethical Clearance Number 2.216/IX/HREC/2024.

Preparation of starfruit leaf simplicia

In this research, starfruit leaves were examined to determine the true identity of the plant using key identification characteristics. Determination was carried out at Muhammadiyah University of Surakarta, with Number 016/A.E-1/LAB.BIO/X/2024. Based on the result, it can be concluded that the plant is *Averrhoa carambola* L.

The material used in this study was young and fresh starfruit leaves taken from Desa Depok, Kecamatan Toroh, Kabupaten Grobogan. After collecting the starfruit leaves, the next step was wet sorting, which involved separating dirt while the leaves were still fresh. After that, the starfruit leaves were heated in the sun for 3 days until the leaves became wilted, turned brown, and became brittle when we crushed.

Preparation of ethanol extract of starfruit leaves

Starfruit leaf simplicia was extracted using the remaceration method. The weight of the simplicia used was 1 kilogram, which was then divided into 3 parts. Maceration was performed using 96% ethanol solvent with a ratio of 1:10 (w/v). During the maceration process, stirring was carried out daily for 15 minutes until the third day. Then the mixture was separated by filtration using filter paper to obtain the filtrate and separated sweet star fruit leaf powder, thus obtaining filtrate I. After that, remaceration was performed on the sweet star fruit leaf powder residue using 96% ethanol with a ratio of 1:5 (w/v) for 2 days, then filtered to obtain filtrate II. Filtrate I and filtrate II were then combined in a tightly closed container. Subsequently, the entire filtrate was concentrated using a vacuum rotary evaporator at a temperature of 60°C until a thick extract was obtained.

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The percent yield of the extract is calculated using the equation:

$$\% \text{ Extract yield} = \frac{\text{Extract weight}}{\text{Simplicia weight}} \times 100$$

Phytochemical screening of the ethanol extract of starfruit leaves

After obtaining the extract, phytochemical tests were subsequently done using qualitative methods. According to Zaky (2021), these phytochemical tests were.

Flavonoid test

After adding 0.5 mg of magnesium powder and 1 mL of HCl to a test tube containing 1 gram of starfruit leaf ethanol extract, heating was continued for 15 minutes. If a red, orange, or yellow precipitate formed, the extract contained flavonoids.

Alkaloid test

Having placed 2 g of starfruit leaf ethanol extract in a test tube, 5 mL of 2 N HCl was added, then followed by heating. After cooling, Mayer's reagent was added. If a yellow or white precipitate occurred, the extract contained alkaloids.

Saponin test

After putting 1 g of starfruit leaf ethanol extract in a test tube, 10 mL of hot water was added. After cooling, vigorous shaking was done for 10 seconds. The extract was positive for saponin content if foam formed with a height of 1-10 cm lasting no less than 10 minutes, and the foam did not disappear with the addition of 1 drop of 2 N HCl.

Tannin test

Boil 1 g of starfruit leaf ethanol extract in 10 mL of distilled water in a test tube for 5 minutes. It was filtered and 3-4 drops of $FeCl_3$ were added until a color change occurred. The formation of blue-green or blue-black color indicated a positive result for tannins.

Gel formulation

Starfruit leaf extract was then formulated into gel preparations using the formulation previously developed by Susianti (2021). The gel was prepared in 3 formulations with varying concentrations of starfruit leaf ethanol extract: 5%, 10%, and 15%.

Table 1 Starfruit Leaf Ethanol Extract Gel Formulation

Materials	Function	K (-)	FI	FII	FIII
Starfruit leaf ethanol extract (%)	Active ingredient	-	5	10	15
CMC-Na (g)	Gelling agent	1,16	1,16	1,16	1,16
Carbopol 940 (g)	Gelling agent	0,34	0,34	0,34	0,34
Trietanolamin (TEA) (g)	Emulgator	0,60	0,60	0,60	0,60
Glycerin (g)	Humectant	3,99	3,99	3,99	3,99
Propilen glikol (g)	Enhancer	2	2	2	2
Metil paraben (g)	Preservative	0,06	0,06	0,06	0,06
Aquades ad (g)	Solvent	30	30	30	30

The preparation of the starfruit leaf ethanol extract topical gel formula was performed by developing CMC-Na in hot water. Carbopol 940 was developed in hot water and the pH was neutralized using triethanolamine (TEA). The next step was mixing the previously developed CMC-Na and stirring until homogeneous; this mixture was referred to as mixture 1. Then methyl paraben was dissolved using a small amount of water in a beaker, propylene glycol and glycerin were added, and stirred until homogeneous; this mixture was referred to as mixture 2. Both mixtures were stirred until a transparent and homogeneous gel mass was obtained. The final step was the addition of sweet star fruit leaf ethanol extract and the remaining distilled water until reaching 30 g, resulting in a homogeneous topical gel preparation (Susianti et al., 2021).

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Evaluation parameters of gel

Organoleptic test

This test aims to identify the quality of the gel preparation, which includes texture, color, and odor (Yusuf et al., 2022).

Homogeneity test

As much as 1 g of the topical gel extract preparation was weighed on transparent glass or an object glass. A good gel preparation will indicate a homogeneous composition (Yusuf et al., 2022).

Spreadability test

One gram of topical gel extract was weighed and positioned at the center of the glass with the diameter of the spreadability test apparatus. The round glass was weighed first, then placed over the glass containing the gel for 1 minute, and the spread diameter was measured. A 50-gram load was added to the glass and left for 1 minute, then the spread diameter was measured. Loads were added up to 150 grams until the gel diameter became constant. The diameter of the spread gel was calculated by measuring it on all four sides (Edityaningrum et al., 2022).

Adhesion test

The researchers weighed 0.5 grams of topical gel extract and placed it at the center of a microscope slide, then covered it with another microscope slide. They added a 1 kg weight on top for 5 minutes. They attached the testing device with an 80-gram load to the microscope slide, then calculated the time needed for both microscope slides to detach (Slamet et al., 2020).

pH test

The pH measurement was carried out using a Hanna pH meter by dipping the device into a gel sample of 500 mg that was dissolved in 50 mL of distilled water, then stirred until evenly mixed. The pH meter was calibrated before use using pH buffers of 4, 7, and 10. After calibration, the pH meter could be used to measure the pH of the gel preparation (Hidayat, 2022).

Viscosity test

The viscometer spindle was dipped into the gel preparation placed in a beaker at the appropriate speed. Spindle number 6 and at a speed of 50 rpm are used for testing the gel preparation. The preparation's viscosity was visible on the instrument's scale section after reaching stability (Slamet et al., 2020).

Burn wound-healing test

The animals were white rats of the Wistar strain, aged 2-3 months, weighing 200-250 grams and acclimated for a week (Siregar & Hariaji, 2021). The test animals on the back were shaved about 3 cm and then anesthetized first using ketamine 0,3 mL intramuscular. An iron plate with a diameter of 2 cm was previously heated for 5 minutes then induced to the rats' backs for 5 seconds. The gel was administered by applying it to the wound on the rat twice a day for 15 days. Test animals were divided into 5 groups, each group consisted of 5 rats. Treatments based on treatment groups were:

1. Positive control (K+) : Bioplacenton®
2. Negative control (K-) : Gel base
3. F1 : Starfruit ethanol extract gel concentration 5%
4. F2 : Starfruit ethanol extract gel concentration 10%
5. F3 : Starfruit ethanol extract gel concentration 15%

Macroscopic observation with Nagaoka score

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Macroscopic evaluation of burn wound healing was monitored for 15 days using the Nagaoka method. Assessment was based on healing duration in days, local infection signs, and local reaction indicators

Table 2 Nagaoka Score

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

This study utilized an observational method with test animals divided into 5 groups and observations were made on days 0, 1, 7, and 14 to evaluate macroscopic signs of wound healing using the Nagaoka score (Nagaoka et al., 2000).

Measurement of burn wound-healing percentage

The measurement of burn wound healing was carried out by measuring the burn wound diameter in experimental animals on day-x using vernier calipers. These measurements were performed on days 1, 5, 10, and 15. The parameter utilized was the burn wound healing percentage on day-x (Leny et al., 2021).

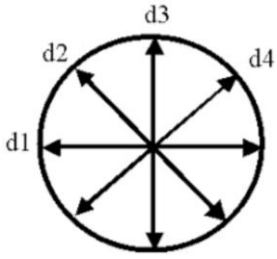

Figure 1 Measurement Of Burn Wound Diameters

The calculation formula for wound diameter is as follows:

$dx = \frac{d1+d2+d3+d4}{4}$

dx = wound diameter at day x
d1 = diameter 1
d2 = diameter 2
d3 = diameter 3
d4 = diameter 4

The formula for calculating burn wound healing percentage is as follows:

$P\% = \frac{(d0)^2-(dx)^2}{(d0)^2}$ x 100%

P% = burn wound healing percentage
d_0 = average baseline wound diameter
d_x = average wound diameter on observation day (day x)

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Data analysis

The obtained research data, consisting of Nagaoka scores and percentages, were analyzed using SPSS version 23. Data analysis started with the Shapiro-Wilk test to determine whether the data followed normal distribution, then homogeneity testing was performed using Levene's test. To identify if at least two groups showed significant differences, a one-way ANOVA was conducted, followed by Tukey's post hoc test (Wardani et al., 2024).

RESULTS AND DISCUSSION

Results of extraction of starfruit leaves

The starfruit ethanol extract has a yield percentage of 26,33% with standard deviation 5,32 as presented in Table 3. The requirement for thick extract yield according to the Indonesian Herbal Pharmacopoeia (2017) is not less than 10%. The yield results obtained meet the requirements and this value indicates that the sweet star fruit leaf extraction process provide consistent results.

Table 3 Results of extraction starfruit leaves

Simplicia (g)	Extract weight (g)	% Yeild
250	73	29,20
250	74	29,60
500	101	20,20
Average±SD		26,33±5,32

Results of phytochemical

Phytochemical testing aims to carry out the identification of active chemical compound content found in the ethanol extract of sweet star fruit leaves. This study tested various secondary metabolite compounds including saponin, alkaloid, tannin, and flavonoid groups. The phytochemical test results of sweet star fruit leaf ethanol extract presented in

Table **4** show that the sweet star fruit leaf ethanol extract contains alkaloids, flavonoids, and saponins. However, it does not contain tannins.

Table 4 Results of phytochemical

Golongan senyawa	Results According to Literature	Results	Notes
Alkaloid	*Mayer*: white precipitate	*Mayer*: white precipitate	+
	Bouchardat: chocolate precipitate	*Bouchardat*: chocolate precipitate	+++
	Dragendorf: yellow precipitate	*Dragendorf*: yellow precipitate	+
Flavonoid	Red, orange, yellow, or yellow color	Red color	++
Saponin	Foam height 1 - 10 cm	Foam formation	++
Tannin	Blue-black, black-green, or black color	No color change occurred	-

Notes:
(-) : does not contain secondary metabolite compounds
(+) : small amount
(++) : moderate amount
(+++) : large amount

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Results of evaluation parameters of gel

Evaluation parameters of gel includes organoleptic, homogeneity, viscosity, pH, spreadability, and adhesion. The result can be seen in the Table 5.

Table 5 Results of Evaluation Parameters of Gel

Parameter	Formula			
	K-	F1	F2	F3
Organoleptic	Transparent	Dark green, distinctive smell and thick	Dark green, distinctive smell and thick	Dark green, distinctive smell and thick
Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous
Viscosity (cPs)	5740 ± 102,89	12273 ± 136,14	13513 ± 100,66	15767 ± 30,55
pH	6,47 ± 0,02	6,37 ± 0,01	6,29 ± 0,01	6,17 ± 0,01
Spreadability (cm)	5,93 ± 0,15	5,40 ± 0,09	5,37 ± 0,08	4,78 ± 0,03
Adhesion (s)	2,27 ± 0,05	2,72 ± 0,05	2,82 ± 0,07	3,32 ± 0,12

Organoleptic

This test relies on the senses to evaluate the color, odor, and form of the gel being tested. Organoleptic testing on three gel formulas with varying amounts of sweet star fruit leaf ethanol extract resulted in darker and more concentrated colors, a characteristic leaf odor, and a thicker gel consistency.

Homogeneity

The homogeneity test conducted aims to assess the level of homogeneity of the gel preparation because homogeneity affects effectiveness in wound healing. It is assumed that a homogeneous gel preparation has an even distribution of active substances so that in the gel preparation it ensures that each part has the same concentration, making its use and application more stable

Viscosity

The viscosity test of the gel preparation uses a *Brookfield Viscometer*. Spindle number 6 is used for testing the gel preparation. The viscosity measurement process is carried out by dipping the spindle into the preparation until it reaches the limit mark on the viscometer, then the device is turned on. The number displayed on the device is the gel viscosity value which is then shown in Table. The test results of topical gel preparations without extract and topical gels containing sweet star fruit leaf ethanol extract are in the range of 5740 – 15767 cPs, which meets the SNI (Indonesian National Standard) requirements for topical gel preparations with viscosity between 3000 – 50000 cPs

pH

Gel preparations must have an appropriate pH within the normal skin pH range of 4.5 – 6.5 (Thomas et al., 2023). If the pH is too low or acidic, it will risk causing skin irritation, and if the pH is too high or alkaline, it can cause dry skin reactions. The test findings for all four preparation formulas meet the gel preparation pH requirements. Therefore, the topical gel preparation of sweet star fruit leaf ethanol extract is safe to use and will not risk irritating the skin.

Spreadability

Gel preparations are expected to spread easily on the application area, so that the wider the contact of active substances with the skin, the more optimal the absorption of these active substances. According to Garg (2002), the consistency indicator for semisolid preparations that

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meets requirements is having a spreadability in the range of 5 – 7 cm. Preparations with lower viscosity will produce greater spreadability. According to Sayuti in Yati (2018), greater spreadability indicates that the active substance has a broader ability to spread and interact with the skin. The spreadability test results in Table 10 are in the range of 4.78 – 5.93 cm. Thus, the spreadability of all formulas has met the requirements, therefore the gel will easily spread effectively on the skin.

Adhesion

The requirement for a good adhesiveness test must show a duration of more than 1 second (Voigt, 1994). In testing the four gel preparations, it was shown that the adhesion duration of the preparations was more than 1 second. Thus, the topical gel of sweet star fruit leaf ethanol extract demonstrates good ability to adhere to the skin surface.

Results of burn wound-healing test

Precentage of Burn Wound Healing

This research aims to determine the effect of varying concentrations of sweet star fruit (Averrhoa carambola) leaf ethanol extract in topical gel formulation on burn wounds. The results of testing the topical gel formulation of sweet star fruit leaf ethanol extract applied to the backs of Wistar strain rats for 15 days can be seen in the Table 6.

Table 6 percentage of burn wound healing

Day	Percentage of Burn Wound Healing ± SD				
	K+	K-	F1	F2	F3
1	0,00±0,00^{bce}	0,00±0,00^{ae}	0,00±0,00^{a}	0,00±0,00^{a}	0,00±0,00^{ab}
2	13,97±6,60^{bce}	10,22±6,62^{ae}	8,37±4,45^{a}	9,61±3,36^{a}	12,70±5,31^{ab}
3	40,11±11,35^{bce}	16,43±5,51^{ae}	14,96±3,45^{a}	20,56±4,63^{a}	23,50±6,64^{ab}
4	59,18±4,87^{bce}	28,35±9,73^{ae}	23,39±4,38^{a}	28,33±6,15^{a}	33,96±5,93^{ab}
5	75,06±5,65^{bce}	32,08±11,25^{ae}	31,17±6,11^{a}	36,31±10,07^{a}	42,51±3,35^{ab}
6	80,05±2,45^{bce}	37,53±11,06^{ae}	37,31±6,33^{a}	41,11±10,45^{a}	48,98±3,15^{ab}
7	83,13±2,29^{bce}	41,22±13,04^{ae}	43,42±7,08^{a}	45,92±9,82^{a}	54,91±3,96^{ab}
8	87,40±2,13^{bce}	45,39±12,63^{ae}	48,69±9,06^{a}	53,78±7,61^{a}	59,60±3,93^{ab}
9	89,53±1,34^{bce}	48,90±11,93^{ae}	52,63±8,47^{a}	57,63±9,10^{a}	64,31±2,89^{ab}
10	92,22±1,47^{bce}	51,60±11,41^{ae}	57,23±7,38^{a}	61,15±9,01^{a}	68,92±2,90^{ab}
11	94,16±0,93^{bce}	53,91±11,23^{ae}	60,00±5,96^{a}	64,58±8,13^{a}	74,02±3,14^{ab}
12	96,27±0,35^{bce}	56,19±10,80^{ae}	64,45±5,18^{a}	68,54±7,15^{a}	77,72±0,72^{ab}
13	98,05±1,00^{bce}	59,96±11,33^{ae}	66,87±5,57^{a}	72,91±4,60^{a}	81,13±1,82^{ab}
14	99,11±1,00^{bce}	62,24±11,66^{ae}	71,23±6,12^{a}	77,28±4,25^{a}	83,25±1,46^{ab}
15	99,78±0,35^{bce}	64,87±10,16^{ae}	75,54±7,52^{a}	80,62±4,17^{a}	86,15±0,90^{ab}

Notes :

a : there is a difference with the positive control
b : there is a difference with the negative control
c : there is a difference with the starfruit ethanol extract gel concentration 5%
d : there is a difference with the starfruit ethanol extract gel concentration 10%
e : there is a difference with the starfruit ethanol extract gel concentration 15%

Table 6 shows that there is a burn wound healing process observed from the burn wound healing percentage data over 15 days. Compared to other treatment groups, the negative control group experience the least improvement. This is because the preparation used by the negative control group does not contain active ingredients that are important for burn wound healing. In this research is a comparison of burn wound healing percentages between groups of Wistar

strain rats given topical gel formulation of sweet star fruit leaf ethanol extract and groups of Wistar strain rats given Bioplacenton formulation.

Macroscopic Observation by Nagaoka Method

Wound healing score

Table 6 Wound healing score

Wound healing time				
Group	**Score**	**n**	**%**	**Score average**
Control +	1	5	100	
	2	0	0	
	3	0	0	
Total		5	100	1
Control -	1	5	100	
	2	0	0	
	3	0	0	
Total		5	100	1
F1	1	5	100	
	2	0	0	
	3	0	0	
Total		5	100	1
F2	1	5	100	
	2	0	0	
	3	0	0	
Total		5	100	1
F3	1	5	100	
	2	0	0	
	3	0	0	
Total		5	100	1

From Table 6, the data shows that all groups have a score of 1 (100%) with an average healing score of 1 across all groups, indicating no difference in healing speed between the control groups and treatment groups and sweet star fruit leaf ethanol extract topical gel formula.

Local infection

Table 7 Local infection

	Local Infection			
Group	**Score**	**n**	**%**	**Score average**
Control +	1	0	0	
	2	0	0	
	3	5	100	
Total		5	100	3
Control -	1	0	0	
	2	1	20	
	3	4	80	
Total		5	100	2,80
Formula 1	1	0	0	
	2	0	0	

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Local Infection				
Group	**Score**	**n**	**%**	**Score average**
	3	5	100	
Total		5	100	3
Formula 2	1	1	20	
	2	0	0	
	3	4	80	
Total		5	100	2,60
Formula 3	1	0	0	
	2	0	0	
	3	5	100	
Total		5	100	3

Based on Table 7 the data show that in the positive control group, formula 1, and formula 3 groups, all samples demonstrate an infection reaction score of 3 (100%) with the same average score of 3. Meanwhile, in the negative control group, there is 1 sample with a score of 2 (20%) and 4 samples with a score of 3 (80%), while in the formula 2 group, there is 1 sample with a score of 1 (20%) and 4 samples with a score of 3 (80%). Based on the average score values, the formula 2 treatment group show the lowest score at 2.60.

Allergic reaction

Allergic Reaction				
Group	**Score**	**n**	**%**	**Score average**
Control +	1	0	0	
	3	5	100	
Total		5	100	3
Control -	1	1	20	
	3	4	80	
Total		5	100	2,60
Formula 1	1	0	0	
	3	5	100	
Total		5	100	3
Formula 2	1	2	40	
	3	3	60	
Total		5	100	2,20
Formula 3	1	0	0	
	3	5	100	
Total		5	100	3

Based on table 15, the data show that in the positive control group, formula 1, and formula 3 groups, all samples demonstrate an infection reaction score of 3 (100%) with the same average score of 3. Meanwhile, in the negative control group, there is 1 sample with a score of 1 (20%) and 4 samples with a score of 3 (80%), while in the formula 2 group, there is

1 sample with a score of 1 (20%) and 4 samples with a score of 3 (80%). Based on the average score values, the formula 2 treatment group show the lowest score at 2.20.

Total score

Table 8 Total score

Total score			
Group	**Skor**	**n**	**Score average**
Control +	5	0	
	6	0	
	7	5	
Total		5	7
Control -	5	1	
	6	1	
	7	3	
Total		5	6,40
Formula 1	5	0	
	6	0	
	7	7	
Total		5	7
Formula 2	5	3	
	6	0	
	7	2	
Total		5	5,80
Formula 3	5	0	
	6	0	
	7	5	
Total		5	7

Based on Table 8 explains that the total healing score in the positive control group, formula 1, and formula 3 all have a total score of 7 across all samples, while in the negative control group, 3 samples have a total score of 7, 1 sample has a total score of 6, and 1 sample has a total score of 5. In the formula 2 treatment group, 3 samples have a total score of 7 and 3 samples have a total score of 5. Based on the average total score values, the formula 2 treatment group show the lowest score at 5.80.

From the five groups, the normality test results using the Shapiro-Wilk test show a p-value of $0.00<0.05$, indicating that the data is not normally distributed. The analysis is then continued using a non-parametric test, namely the Kruskal-Wallis test, to compare differences in Nagaoka scores between groups, which show results of $p=0.043<0.05$, meaning there are significant differences in Nagaoka scores between research groups. To determine which groups have significant differences, a post hoc Mann-Whitney U test is conducted by comparing the F2 group, which have the lowest mean rank of 7.70, with the other research groups. The results of the Mann-Whitney U test show that the F2 group have significantly lower Nagaoka scores when compared to the K+, F1, and F3 groups, but is not significantly different from the K- group.

Based on the data on burn wound healing percentage and the Nagaoka scoring method, it can be seen that formula 3 shows a good percentage of burn wound healing compared to other groups. In the Nagaoka method, it is also observed that formula 3 demonstrates a good healing time score and no irritation or allergic reactions occur in the formula 3 group.

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The optimal burn wound healing is found in formula 3 with a starfruit leaf extract concentration of 15%, which contains a greater variety of compounds capable of healing burn wounds. The compounds contain in star fruit leaf extract including flavonoids, which have been proven to have potential as anti-inflammatory agents with diverse mechanisms of action, including inhibiting the activity of enzymes and transcription factors involved in regulating inflammatory mediators (Sanjaya et al., 2023). Alkaloids have antimicrobial properties by disrupting peptidoglycan components in bacterial cells, causing the cell wall layer can't form completely, resulting in cell death (Athandau et al., 2023). Saponins function as antiseptics by stimulating epidermal cell proliferation, accelerating keratinocyte migration to the wound area, and stimulating type I collagen production (Sani K et al., 2025).

The advantage of gel dosage forms is their cooling effect caused by high water content, so that substance penetration into tissues becomes better. Gel is a pharmaceutical preparation formulated to have the advantages of being non-sticky and easy to wash off, so that the wound healing process can be faster (Kaban et al., 2022).

CONCLUSION

The starfruit leaf extract gel has burn wound healing ability for second-degree burns in Wistar strain rats. Based on analysis using wound healing percentage and Nagaoka scores, the optimal concentration was 15% with healing time equivalent to the positive control group.

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AUTHOR'S DECLARATION

Authors' contributions and responsibilities

All authors made substantial contributions to the conception and design of the study; took responsibility for data analysis, interpretation and discussion of results; and read and approved the final manuscript.

Availability of data and materials

All data are available from the authors

Competing interests

The authors declare no competing interest

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