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Characteristics of hibiscus (*Hibiscus rosa-sinensis* L.) leaf extract gel and effectiveness in healing burn wounds in rats (*Rattus norvegicus* L.)

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ABSTRACT

This study aimed to determine the physicochemical characteristics of hibiscus leaf extract gel and elucidate its effectiveness on burn wound healing on the back of rats (Rattus norvegicus L.) wistar strain. This research was done by the Complete Randomized Design with 5 treatments and 4 repetitions, namely Negative Control (Gel base), Positive Control (Bioplacenton 10%), G1 (2% hibiscus leaf extract gel), G2 (4% hibiscus leaf extract gel) and G3 (6% hibiscus leaf extract gel). The physicochemical characteristics of the gel were determined including organoleptic test,, homogeneity, pH, viscosity and spreadability. Meanwhile, the burning on the rat's back was carried out using a hot metal coin with a diameter of 20 mm that attached to the rat's back. Data on wound healing effectiveness parameters were analyzed using One Way ANOVA (α = 0.05) and advanced Least Significant Different (LSD) test. The results showed that the organoleptic test did not meet the requirements of gel preparations in terms of color and aroma, while the homogeneity, pH, viscosity and spreadability tests met the requirements of gel preparations. Hibiscus leaf extract gel at 6% was found more effective in accelerating the healing of burn wounds which has the most optimal healing power with an average wound healing time of 15.75 days and a healing percentage of 97.71%, as compared with Bioplacenton 10% followed by 4% and 2% extract gels.

Keywords : Healing of burns effectiveness, Hibiscus rosa-sinensis extract. Rattus norvegicus

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INTRODUCTION

Burns are injuries to the outer skin layer and deeper tissues caused by exposure to fire, chemicals, electricity, radiation and radioactivity (Nofiyanto & Nirmalasari, 2020). The depth of a skin burn is determined by several factors, namely the high temperature that caused the injury, the thickness of the skin and the length of time the skin is exposed to substances or objects that cause burns, which can affect wound healing time (Christie *et al*., 2018). The proportion of burn patients in Indonesia reached 1.3% of the population with the highest number of patients occurring in the age range of 25-34 years (Ministry of Health of the Republic of Indonesia, 2018), and therefore it needs practices solution to preven further skin burn happening.

One of the appropriate treatments for burn patients is to treat the wound by using topical drugs in the form of gel, cream or ointment preparations (Fitriani *et al*., 2022). Gel is a dosage form that is often chosen in the treatment of burns. This is because the gel has the advantage of being easily absorbed into the skin, giving a cold reaction because it contains a high amount of water and is bound by humectants so that it can provide moisture to the wound, is not sticky and comfortable to use and has a good drug release so that it can accelerate wound healing. A commonly used synthesized gel medication for treating burns is Bioplacenton (Muthmaina *et al*., 2017). The use of Bioplacenton has side effects such as hypersensitivity to the skin in the form of dermatitis and the content of neomycin

sulfate can cause side effects such as itching, swelling and irritation (Sunardi *et al*., 2019). Meanwhile, the side effects caused by these synthetic drugs cause people to tend to switch to using herbal medicines derived from natural ingredients to treat burns.

Plants that can be used as medicine to heal burns are hibiscus leaves, which have used by the community to treat wounds, boils, fever, rheumatism, kidney and skin inflammation. Hibiscus leaves have been used for generations as a traditional medicine to treat infectious wounds (Yamlean & Lolo, 2016). *Hibiscus rosa-sinensis* L. leaves contain secondary metabolite compounds such as saponins, phenols, alkaloids, tannins, flavonoids and terpenoids (Paul *et al*., 2016). Secondary metabolite compounds that play an important role in healing wounds are flavonoids and saponins. Flavonoid levels in hibiscus leaves amounted to 24.98 mgQE/g (Sharma & Thakur, 2022). Flavonoids in healing wounds can increase antioxidant activity in granuloma tissue and function as anti-inflammatory while saponins play a role in the wound contraction process and increase the epithelialization process and have antimicrobial activity (Nuralifah *et al*., 2022). Hibiscus leaves contain biopolymers in the form of mucus-shaped polysaccharides which are one of the components of thickeners or gelling agents so that they have a texture that is almost the same as a gel (Kartika *et al*., 2021).

Although previous studies has elucidated the utilization of hibiscus plants in wound healing (Gunawan 2016, Yamlean & Lolo 2016, Bruan & Tianco 2019), there were still unclear how the hibiscus leaf extract could be used by community. This study aimed to determine the physicochemical characteristics of hibiscus leaf extract gel and its effectiveness on burn healing on the back of rats (*Rattus norvegicus* L.) Wistar strain.

RESEARCH METHODS

This type of research is experimental, which presented in the Table 1. The research population and samples were 20 male Wistar rats (*Rattus norvegicus* L.) weighing 120-200 grams aged 2 months. Mice were divided into 5 treatments and each consisted of 4 repetitions, namely negative control (KN), positive control (KP) gel extract treatment with a concentration of 2% (G1), concentration of 4% (G2), and concentration of 6% (G3). 300 grams of hibiscus leaves were macerated with 96% ethanol for 2 x 24 hours. The sample is filtered and the filtrate is concentrated using a rotary evaporator to obtain a thick extract.

Table 1. Hibiscus leaf ethanol extract gel formula (*Hibiscus rosa-sinensis* L.)

No	Material	Concentration (%)		
		G1	G2	G3
1	Ethanol extract of hibiscus leaves	2	4	6
2	Glycerin	10	10	10
3	Propylene glycol	5	5	5
4	Na-CMC	3	3	3
5	DMDM Hydantoin	0,5	0,5	0,5
6	Aquadest	Ad 100	Ad 100	Ad 100

Preparation of Hibiscus Leaf Extract

Fresh hibiscus leaves as much as ± 4 kg were washed with water and dried using an oven at 60°C for 2 days. The dried samples were sorted again and continued by smoothing the samples using a blender to form simplisia powder following the method of Rivai *et al*. (2014). Simplisia was then weighed as much as 300 grams and put into a maceration container and given 96% ethanol solvent as much as 3 liters until the simplisia was completely submerged. The maceration container was closed and kept for 2 x 24 hours in a place protected from sunlight and stirred every 6 hours. The sample was then filtered to separate the pulp and filtrate. The filtrate is then concentrated using a rotary evaporator until a thick ethanol extract is obtained (Indriani *et al*., 2020).

Preparation and Evaluation of Hibiscus Leaf Extract Gel Preparation

The gel formula was made by weighing the ingredients according to the formulation. Before making the gel, distilled water is put into a glass beaker and then heated on a hot plate with a temperature of 900C until hot, then put Na-CMC into a mortar and add 10 mL of heated distilled water and stir until it melts without any white grains. The next step includes propylene glycol,

glycerin and DMDM Hydantoin while stirring. Next, enter the hibiscus leaf extract that has been prepared and add the remaining distilled water, then stir at a constant speed until homogeneous (Indriani *et al*., 2020).

Gel characteristics of hibiscus leaf extract

Organoleptic test

Organoleptic test is done visually and seen directly the shape, color, smell, of the gel made. The requirements for gel preparations have a semi-solid shape, clear color and do not have a pungent odor (Slamet *et al*., 2020)**.**

Homogeneity test

The homogeneity test is carried out by applying a gel sample to a piece of glass or other suitable transparent material, the preparation should show a homogeneous composition and no coarse grains should be visible (Slamet *et al*., 2020)**.**

pH test

The pH test is carried out to see the acidity of the preparation so that the preparation does not cause irritation to the skin. Gel preparations are tested for pH using a pH meter, by dipping the pH meter stick into the preparation. The results of the pH value appear on the pH meter screen monitor (Chandra & Rahmah, 2022). The pH value of the optimal preparation should be close to the pH of the skin, which is 4.5 - 6.5 (Astuti *et al*., 2017)

Viscosity test

The viscosity test was conducted using a Rion viscometer. The tool is prepared in a horizontal position and the rotor can be adjusted so that the pointer needle is exactly horizontal, inserting a sample of 50 grams into the viscometer cup. The rotor was inserted into the cup until the limit indicated on the rotor. The viscometer is then turned on so that the rotor will begin to rotate and wait a few moments for the needle to stabilize (Fahrezi *et al*., 2021). The viscosity requirement for gel preparations is 3000 to 50,000 cP.

Spreadability test

This test is carried out by weighing 0.5 grams of gel then placed in a round glass, another glass is placed on top and left for 1 minute. After that, 150 grams of load was added and left for 1 minute and the constant diameter was measured. The qualified spreadability is 5-7 cm (Slamet *et al*., 2020).

Burn treatment and creation

Rats were shaved the day before the burns were made on the back area. Making burns begins with giving topical anesthesia using ethyl chloride spray on the back that will be given a burn. The rat's back that has been shaved is cleaned using 70% alcohol, then burns are made on the rat's back using a 20 mm diameter Rp.100 metal coin that has been heated in a bunsen flame for 5 minutes. The heated coin was then affixed for 2 seconds to the rat's back until a burn of degree II A was formed, characterized by a reddish color and the formation of ulcers (water bubbles) on the rat's back (Sudarko, 2018).

The administration of hibiscus leaf extract gel to burn wounds in each treatment was carried out by applying 200 mg of hibiscus leaf extract gel using a cotton bud to the wound topically by applying 3 times a day in the morning, afternoon and evening. Measurement of burn wound diameter in each test animal was carried out once a day before gel application for 18 days to observe the effectiveness of each treatment group using the morton method. Wound application using Bioplacenton 10% was also done at the same time as the application of hibiscus leaf extract (Rustian *et al*., 2022).

Data Analysis

Data in this study was conducted using the Statistical Product Services Solution (SPSS) application program version 23.0. Data on burn wound healing parameters were statistically analyzed using one way ANOVA with 95% confidence level to determine the effect of hibiscus leaf extract gel on burn wound healing and continued with LSD (Least Significant Different) test with 95% confidence level

RESULTS AND DISCUSSION

Gel Characteristics of Hibiscus Leaf Extract

The phytochemical characteristics of gel under different Hibiscus leaves's extract is presented in the Table 2.

Table 2. Gel characteristics of hibiscus leaf extract

Physicochemical Characteristics	KN	G1	G2	G3
Organoleptic				
Texture	Semi solid	Semi solid	Semi solid	Semi solid
Color	Clear	Green	Green brown	Blackish green
Odor	No odor	typical hibiscus leaves	typical hibiscus leaves	typical hibiscus leaves
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH	6,42	6,16	5,85	5,34
Viscosity	3.500 Cp	5.000 cP	7.800 cP	10.000 Cp
Spreadability	6,5 cm	6,28 cm	5,68 cm	5,42 cm

The results of organoleptic observations of gel preparations of ethanol extract of hibiscus leaves seen from the texture of the preparations of the four formulas have a semi-solid texture. The preparation is said to be semi-solid because it has characteristics between solid and liquid so that when the container is turned over, the gel will flow slowly but still looks like a solid. According to Kusuma *et al*. (2017) semi-solid preparations such as gels have tiksotropic flow properties, namely when given a force it will flow slowly and when the force is removed the preparation will solidify again. The color of the preparation and odor in formula KN is colorless or clear and has no odor, 2% gel (G1) has a green color, 4% gel (G2) and 6% gel (G3) have a blackish green color with a distinctive smell of hibiscus leaf extract. The addition of hibiscus leaf extract causes the color of the preparation to become more concentrated and does not appear clear and causes a pungent hibiscus leaf odor so that it does not meet the organoleptic test parameters of the gel preparation in terms of color and odor. The homogeneity test results showed that in the four gel formulas produced in this study based on the observation results there were no lumps or coarse particles in the gel, the color of the preparation was evenly distributed so that it could be said that the four gel formulas were homogeneous and met the homogeneity requirements for gel preparations.

The results of the pH test of gel preparations, where the four gel formulas produced in this study have a pH value that is in accordance with the pH value of the skin, namely 4.5 - 6.5, so it can be said that the four formulas meet the pH requirements of gel preparations. The pH test in this study shows that the addition of ethanol extract of hibiscus leaves can affect the pH of the preparation, where the higher the concentration of extract given, the lower the pH of the gel preparation. This is because the extract is acidic so it can lower the pH of the gel preparation. According to Kusuma *et al*. (2017), the extract is acidic because it contains secondary metabolite compounds such as flavonoids, saponins, phenols which are acidic due to the nature of the –OH group which easily releases H+ ions.

The viscosity test results showed that the four gel formulas met the requirements for good gel viscosity. Increasing the concentration of ethanol extract of hibiscus leaves added to the gel preparation can increase the viscosity of the preparation. This is due to the thick and sticky texture of the extract so that when added to the preparation it will increase the viscosity or viscosity of the gel preparation. This is in accordance with research from Indriani et al. (2020) which shows that the higher the concentration of ethanol extract of red shoot leaves added to the gel preparation, the more the viscosity of the gel preparation will increase. The viscosity requirement for gel preparations is 3,000 to 50,000 cP (Chandra & Rahmah, 2022).

The results of testing the spreadability of gel preparations of the four formulas show that the spreadability of the preparation will decrease as the concentration of ethanol extract of hibiscus leaves added to the preparation increases. This is because the greater the concentration of the extract, the greater the viscosity of the preparation so that the spreadability of the gel will be smaller (Aprilianti *et al*., 2020). Good spreading ability will make it easier to apply on the surface of the skin, the distribution of active substances on the skin will be more even so that the effects caused by the active

substances in the gel preparation will be more optimal. The gel spreadability test met the requirements for gel spreadability, namely 5-7 cm (Irianto et al., 2020).

Effectiveness of Hibiscus Leaf Ethanol Extract Gel on Burn Wound Healing

Length of burn healing time

The results of observations on the length of time of burn wound healing on the back of rats are presented in Table 3

Table 3. Mean Length of Burn Healing Time in Mice after Hibiscus Leaf Extract Gel Administration

Treatment	Average ±SD
KN	18 ± 0.00^{a}
KP	16 ± 0.00^{cd}
G_1	17.25 ± 0.50^{b}
G_2	16.5 ± 0.58^{c}
G_3	15.75 ± 0.50^{d}

Note: Numbers followed by different letters indicate significant differences based on the LSD test (α=0.05)

Based on Table 3, it shows that there are differences in the healing time for burn wounds in each treatment. The fastest healing time for burn wounds occurred in the G_3 treatment (Gel 6%) with an average wound healing time of 15.75 days. The KP treatment has a longer healing time than G_3 (3% Gel) with an average wound healing time of 16 days, but is faster than the G_1 (2% Gel) and G_2 (4% Gel) treatments with an average wound healing time of 17.25 days and 16.5 days. The KN treatment has a slower healing time, namely 18 days. The wound healing process in each treatment was influenced by the concentration of hibiscus leaf ethanol extract given in the preparation. The higher the concentration of the extract in the gel preparation formulation, the faster the wound healing will occur. This is in accordance with the statement from Mayefis et al (2020) which states that the higher the concentration of gotu kola and aloe vera herbal extracts given in the preparation, the faster the wound healing process will be.

Based on the results that active compounds contained in ethanol extract of hibiscus leaves in gel preparation formulas such as flavonoids and saponins can accelerate wound healing (Mayefis *et al* (2020). The active compounds in the gel preparation will be trapped in the gel matrix in the form of a mesh or three-dimensional network formed due to the interaction between polymers in the form of Na-CMC and water (Silvia *et al*., 2021). The active compound will then be released when applied to the wound so that it can accelerate the wound healing process. This is different from the KN which does not contain ethanol extract of hibiscus leaves so that there are no active compounds that can help the wound healing process and the healing process runs slower than the other treatments.

Burn Healing Percentage

The results of the average percentage of burn wound healing are presented in Table 4 below.

Table 4. Mean Percentage of Burn Wound Healing on the Rat's Back between Treatments of Hibiscus Leaf Ethanol Extract Gel

Treatment	Healing Percentage (%)			
	Day 1	Day 6	**Day 12**	**Day 18**
KN (-)	0	$12,22\pm0,17^{c}$	$40,38\pm0,60^{d}$	$88,78\pm1,21^{c}$
KP (+)	0	$13,81\pm0,69^{b}$	$51,45\pm2,53^{ab}$	$97,45\pm0,34^{a}$
G_1	0	$12,41\pm0,39^{c}$	$45,30\pm2,27^{c}$	$94,73\pm0,67^{b}$
G_2	0	$14,12\pm0,54^{b}$	$50,17\pm2,19^{b}$	$95,58\pm0,45^{b}$
G_3	0	$15,58\pm1,28^{a}$	$54,01\pm2,73^{a}$	$97,71\pm0,47^{a}$

Note: Numbers followed by different letters indicate significant differences based on the LSD test (α=0.05)

The high percentage of 6% gel burn wound healing on days 6, 12 and 18 was due to the fact that there was no infection caused by the entry of pathogenic bacteria into the wound, one of which was *Staphylococcus aureus* bacteria. Wounds that experience bacterial infection can prolong the inflammatory process because the body's response to bacteria that enter the body is to release substances such as cytokines, prostaglandins and histamine to fight bacteria that enter the body (Sayogo *et al*., 2017). This causes the inflammatory period to get longer, so that the components needed to produce new tissues are inhibited from forming. Research from Sari & Islamulyadin (2018) states that hibiscus leaf juice is effective in inhibiting the growth of Staphylococcus aureus bacteria by forming an inhibition zone on the media so that it can be said that ethanol extract of hibiscus leaves has antibacterial activity in the wound healing process. According to Egra *et al.* (2019) flavonoids act as antibacterials by damaging the bacterial cell membrane by breaking peptidoglycan bonds so that the selective permeability function and active transport function are disrupted, causing bacteria to lose shape and lysis. Flavonoids can also inhibit the movement of bacteria so as to prevent bacteria from attaching to host cells or colonizing with other bacterial cells, inhibit the release of bacterial toxins and cause damage to the bacterial DNA chain so as to inhibit its growth (Fitriani *et al*., 2022) .

CONCLUSION

The conclusion of this research is that the physicochemical properties of the hibiscus leaf ethanol extract gel formulas I, II and III have a semi-solid texture, blackish green color in the homogeneity test of the homogeneous formula. The pH values are 6.16 (G1), 5.85 (G2) and 5.34 (G3) while the viscosity values are 5,000 cP (G1), 7,800 cP (G2) and 10,000 cP (G3). The dispersion power of the formula is 6.28 cm (G1), 5.68 cm (G2) and 5.42 cm (G2). Hibiscus rosa-sinensis L. leaf ethanol extract gel is effective in healing burns with a concentration of 6% which has the most optimal healing power with an average wound healing time of 15.75 days and a healing percentage of 97.71%. Hibiscus leaf ethanol extract gel can be used as an alternative to speed up the healing of burn wounds.

The conclusion of this research is that the characteristics of the ethanol extract gel preparation of hibiscus leaves (Hibiscus rosa-sinensis L.) have a semi-solid texture, have a blackish green color with a typical extract odor so they do not meet the organoleptic test parameters of the gel preparation in terms of color and aroma. The homogeneity test results meet the requirements for gel preparations, having a pH value that corresponds to the skin pH value, namely 4.5 – 6.5. The viscosity test results meet the requirements for good gel viscosity, and meet the requirements for gel spreadability, namely 5-7 cm. Hibiscus rosa-sinensis L. ethanol extract gel effectively heals burns with a concentration of 6% which has the most optimal healing power with an average wound healing time of 15.75 days and a healing percentage of 97.71%. Hibiscus leaf ethanol extract gel can be used as an alternative to speed up the healing of burn wounds.

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