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Formulation and Activity Test of Nanogel from Ethanol Extract of Breadfulness Leaves (*Artocarpus Altilis*) as A Topical Preparation for Healing Burn Wounds in Male White Rats

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Abstract

Breadfruit leaves (*Artocarpus altilis*) contain secondary metabolites that help burn wound healing, and nanogels improve drug delivery through better skin penetration. This study aims to evaluate the effectiveness of breadfruit leaf ethanol extract nanogel in healing burns in rats. Ethanol extract of breadfruit leaves was obtained by maceration using 96 % ethanol solvent, nanogels were prepared in 3 concentrations namely F1 (1.5625 %), F2 (3.125 %) and F3 (6.25 %) which were tested for physical characteristics of the preparation, stability, antibacterial activity, irritation and burn healing on rat skin. The ethanol extract of breadfruit leaf nanogel was stable for 12 weeks of storage, organoleptically good, good spreadability 5 - 6.1 cm, transmittance percentage test 92.17 - 92.98 %, and more stable at room temperature for 12 weeks of storage, pH stable for 12 weeks of storage, good viscosity between 2,970 - 3,227 cPs, centrifugation test and cycle test showed no phase separation, had antibacterial activity with an inhibition zone diameter of 9.4 to 11 mm against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and did not cause irritation in rabbits. Group F2 showed a faster percentage of wound diameter reduction of 92.84 %, compared to groups F1 and F3 with a healing percentage of 81.14 and 88.91 % concentration within 21 days. Histopathologically, the calculation of the number of angiogenesis, fibroblasts and collagen using a Zeiss Primo Star microscope, Germany, F2 showed better blood vessel formation, which was as many as 14 compared to F1 and F3 with an average of 9.4 and 10 respectively. The formation of the number of fibroblast cells in F1, F2 and F3 was 95, 148 and 132 respectively. Collagen density in F1, F2 and F3 averaged 1 (< 10 %), 3 (50 - 90 %), 3 (90 - 100 %). It can be concluded that F2 has the best healing percentage compared to F1 and F3.

Keywords: Infection, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Artocarpus altilis*, Nanogel

Introduction

Burns are tissue damage that occurs on the surface of the skin, causing inflammation known as erythema [1]. Burns if not treated properly can damage skin tissue. It can also cause irritation and infection of the skin. As a result, the healing of the burn wound becomes longer [2]. Burns are a form of injury that requires good and appropriate treatment. Burns can occur due to direct or indirect fire, either sunlight or chemicals. These conditions can cause the development of pathogenesis in burn wounds and require a long time for skin tissue to close back to heal [3]. Skin is very easily scratched or injured, leading to infection. Infections can be caused by both Gram-positive and Gram-negative pathogenic microbes. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are pathogenic bacteria that often infect human skin [4]. Handling skin infections can be done with the use of antimicrobials. However, irrational use of antimicrobials will trigger the emergence of resistant microorganisms. Antimicrobial resistance is a threat to global public health due to excessive or inappropriate use of antibiotics resulting in ineffective treatment of infections and increased mortality, therefore treatment

using active substances from natural materials is an alternative treatment that is applied because natural materials often contain bioactive compounds that have antimicrobial properties and are not involved in the development of the same resistance as synthetic drugs [5].

One of the plants that has efficacy in healing burns due to the content of secondary metabolites contained in it is breadfruit leaves. Breadfruit (*Artocarpus altilis*) is one of the main fruit-producing plants of the Moraceae family. *Artocarpus altilis* is a multifunctional plant because it is used as a traditional medicine. The utilisation of plants as traditional medicine is related to their bioactive compounds or secondary metabolites. Breadfruit leaves contain flavonoid compounds (anti-inflammatory, antibacterial), tannins (antibacterial), saponins (antibacterial) and polyphenols (antiseptic) [6].

Therefore, it is necessary to make a formula with the active ingredient breadfruit leaf extract in healing burns. Nanogel is one of the topical preparations that is widely chosen because it is easily applied evenly on the skin without pressure, gives a cold sensation, does not leave marks on the skin and is easy to use [7]. Nanogels are nano-sized cross-linked polymer networks that can absorb large amounts of water, nanogels have the ability to respond to biomedically relevant changes such as pH, temperature, in a matter of decades [8]. According to Kurniawan [9], the administration of breadfruit leaf extract gel (*Artocarpus altilis*) can accelerate the healing process of burn wounds in rats at concentrations of 6.25 and 12.5 %. In this study, the authors will make nanogel preparations where some of the objectives are to reduce the concentration of active substances so as to reduce the risk of toxicity while maintaining therapeutic effectiveness. In addition, nanogels with nanometre-scale particle size can release active substances in a controlled manner and facilitate penetration into the skin. This is the basis for the author in using a smaller concentration and is a multiple of the concentration number from the research conducted by Kurniawan [9]. Therefore, the author wants to compare whether the breadfruit leaf ethanol extract nanogel with a smaller concentration than the previous study has good stability at low temperature, room temperature and high temperature, and to see the burn healing activity with a smaller concentration [9].

Materials and methods

Instruments and materials

The instruments employed in this research were digital balance (Dickson), rotary evaporator (Stuart), blender (Miyako), porcelain exchanger, porcelain cup, test tube, petri dish, autoclave, oven, bacterial incubator, disc paper, refrigerator, mortar and pestle, centrifuge (Hitachi CF 16 R X II), magnetic stirrer (E-Liquid), particle size analyzer (Fritch), UV-VIS spectrophotometer, filter paper, digital caliper (Hardened), pH meter (Milwaukee), viscometer (NDJ-8S), homogenizer (IKA), and climate chamber (Memmert), hot metal plate 210 mm^2 . The materials used in this study were breadfruit leaves obtained from Nanggroe Aceh Darussalam, Test bacteria; Staphylococcus aureus (SA), and Pseudomonas aeruginosa (PA), chloral hydrate , Aqua distillata, DMSO (Dimethyl Sulfoxide), Ethanol 96 %, aqueous HCl, McFarland's solution, NaCl 0.9 %, Nutrient agar (OXOID), Carbopol (Ashland), Propylene glycol (USP Grade), Methyl paraben, Propyl paraben (Golden Era), TEA (triethanolamine), Tween 80, neutral pH buffer solution, acid pH buffer solution, Ketamine HCL, Bioplacenton, Formalin 10 %, Liquid Paraffin, Hematoxylin eosin (HE).

Type and location of research

The method used in this research is the experimental method. includes taking and processing samples, making breadfruit leaf simplisia, making ethanol extract of breadfruit leaves, testing the antibacterial activity of extracts, making nanogel formulas, measuring particle size and testing the antibacterial activity of nanogel preparations. The study was conducted at the Phytochemistry, Microbiology and Physical Pharmacy Laboratory of the Universitas Sumatera Utara.

Research procedure

Macroscopic examination

Macroscopic examination is carried out by observing the leaves including a pointed leaf base, rough upper and lower surfaces, pinnate leaf veins, leaf

veins and leaf vein branches appear prominent on the lower surface, rolling to the upper surface.

Microscopic examination

Microscopic examination was carried out on breadfruit (*Artocarpus altilis*) leaf simplisia powder. The simplisia powder was dripped a little on a glass object that had been dripped with chloral hydrate and covered with a cover glass, then observed under a microscope.

Preparation of breadfruit leaf simplicia

The collection of breadfruit leaves was carried out by subjective sampling, the breadfruit leaves that had been collected were washed thoroughly with running water, drained and then weighed the net weight, then dried in a drying cabinet at 40 °C for 3 days. Furthermore, the dried breadfruit leaves were ground using a blender until they became fine powder.

Process of making breadfruit leaf ethanol extract

Weigh 500 g of breadfruit leaf simplisia powder into a closed container, then add 5 L of 96 % ethanol solvent and stir for the first 6 h. Let stand for 18 h while occasionally stirring. Filter using filter paper, collect the filtrate (maserat I). Repeat the extraction process on the pulp using 2.5 L of 96 % ethanol until maserat II is obtained. Combine the 2 macerates. Evaporate the maserat using a Rotavapor device at 40 °C until a thick extract is obtained [7].

Testing the antibacterial activity of ethanol extract of breadfruit leaves

Antibacterial activity test of ethanol extract of breadfruit leaves using the diffusion method. The results of the antibacterial test on the measurement of bacterial growth formed around the disc paper by measuring the diameter of the inhibition zone. Each at a concentration of 1.5625 % (15.265 mg/mL), 3.125 % (31.25 mg/mL), 6.25 % (62.5 mg/mL), 12.5 % (125 mg/mL), 25 % (250 mg/mL), 50 % (500 mg/mL), 100 % (1,000 mg/mL) was dropped on the disc as much as 30 µL. The test bacterial suspension was inserted into Nutrient Agar (NA) media. On the media that had solidified, filter paper that had been soaked (± 15 min) in the test solution of ethanol extract of breadfruit leaves with various concentrations was placed. Incubated at 36 - 37 °C for 24 h, then measured the diameter of the inhibition zone (clear zone) using a digital caliper expressed in millimeters. The test was repeated 3 times [8]. The criteria for selecting concentrations in making formulas are 3 concentrations that have strong inhibitory power and concentrations that are smaller than previous research conducted by Kurniawan [9].

Formulation of breadfruit leaf ethanol extract nanogel

The formulation of the ethanol extract nanogel preparation of breadfruit leaves can be seen in **Table 1**.

Table 1 Formulation of the ethanol extract nanogel preparation of breadfruit leaves to be made.

Components	Percentage of ingredients (%)		
	Formula I	**Formula II**	**Formula III**
Ethanol extract of breadfruit leaves	1.5625	3.125	6.25
Carbopol 940	0.5	0.5	0.5
Tweens 80	0.2	0.2	0,2
Propylene Glycol	4	4	4
Ethanol	3	3	3
TEA	0.3	0.3	0.3
Methyl Paraben	0.2	0.2	0.2
Propyl Paraben	0.02	0.02	0.02
Aquades	Ad 100	Ad 100	Ad 100

Measurement of nanogel preparation particles

Testing to determine particle size was carried out using a particle size analyzer (PSA) by diluting 500 mg of nanogel preparation with 1 mL of distilled water. Then 1 mL of the preparation was taken to test the particle size at room temperature. Observations were made on the breadfruit leaf extract nanogel after weeks 0, 6, and 12 [10].

Organoleptic test and homogeneity of nanogel

Organoleptic tests are carried out by observing the shape, color, and odor of the nanogel preparation. The homogeneity test of the nanogel preparation is applied to a piece of glass or other suitable transparent material, then the preparation must show a homogeneous composition and no coarse grains are visible [7].

Spreadability test of preparation

The examination of the spreadability of the preparation was carried out using a 20×20 cm glass. The measurement was carried out by placing 1 g of the preparation in the middle of the glass, leaving it for 60 s and then measuring the diameter formed. Then it was covered with mica plastic and given a weight load of 0, 50, 100, and 125 g [7].

Transmittance test

The transmittance percentage test was carried out by diluting 0.1 mL of the ethanol extract of breadfruit leaves nanoemulgel to 100 mL using distilled water. The transmittance percentage measurement was carried out at a wavelength of 650 nm using distilled water as a blank. The measurement was carried out using a UV-Vis spectrophotometer [11].

Stability test

Stability test at low temperature (4 ± 2 °C), room temperature (25 ± 2 °C), high temperature (40 ± 2 °C) during 12 weeks of storage, in addition [7].

pH test

The determination of the pH of the nanogel preparation was carried out using a pH meter. The tool was first calibrated using a neutral pH buffer solution and an acidic pH buffer solution until the tool showed the pH value. Then the electrode was washed with distilled water, dried with tissue. The sample was weighed 1 g of the preparation and dissolved with distilled water up to 100 mL. Then the electrode was dipped in the solution and left until the tool showed a constant pH value [12].

Viscosity test

The viscosity examination of the preparation was carried out using an NDJ-8S viscometer by weighing 100 g of the preparation, then setting the spindle 4 and its speed, the NDJ-8S viscometer was run [7].

Centrifugation test

Centrifugation testing of breadfruit leaf ethanol extract nanogel was carried out by inserting the test preparation into a centrifugator with a speed created using centrifugation at a speed of 5,000 rpm for 30 min and observing the phase separation [13].

Cycling test

Cycling test was carried out by storing at a temperature of 4 ± 2 °C for 24 h then removed and stored in a high temperature climatic chamber 40 ± 2 °C for 24 h. This treatment is 1 cycle, the experiment was repeated 6 cycles [14].

Antibacterial activity testing of breadfruit leaf ethanol extract nanogel

Antibacterial activity testing was conducted on breadfruit leaf ethanol extract nanogels with 3 best concentrations. This test was conducted using the agar diffusion method. A total of 0.1 mL of inoculum was put into a sterile Petri dish, after which 15 mL of nutrient agar (NA) media was poured at a temperature of 45 - 50 °C, then the cup was shaken on the surface of the table so that the media and bacterial suspension were evenly mixed and solidified. Each Petri dish was placed with a paper backer that had been dripped with a nanogel test solution of ethanol extract of breadfruit leaves. Then incubated at a temperature of 36 - 37 °C for 24 h, then measured the diameter of the inhibition zone with a caliper expressed in millimetres. The test was conducted with 3 repetitions [8].

Animal

Based on the calculation of the number of samples with the Federer formula, each group contains 5 male white rats, so 25 heads are needed for 5 treatment groups. The test animals used were white rats (Rattus norvegicus) Wistar strain, male, aged about 3 - 4 months, body weight 180 - 200 g, healthy, actively moving, clean and smooth fur, clear eyes, no abnormal discharge from the eyes, ears, anus and did not have any defects and did not experience weight loss of up to 10 % during the acclimatisation period. Acclimatisation of rats was carried out for 7 days. The ambient temperature of the rat cage was room temperature at 25 ± 2 °C. Before burning using an iron plate with an area of 210 mm² heated at 100 °C, the rats were anaesthetised using Ketamine HCL at a dose that had been converted to match the body weight of the rats. Ethical approval for animal care and testing (No.0525/KEPH-FMIPA/2024) was granted by the Animal Ethics Committee of the University of Sumatera Utara.

Irritation test

The skin irritation test was conducted using rabbits. Rabbits were used with the following treatment: Before testing, animals were acclimatised to laboratory conditions for 1 week. The rabbit's back was cleaned. The preparation was applied to the skin area and then covered with gauze plasters. The test was performed on healthy skin. This preparation is a nanogel preparation of ethanol extract of breadfruit leaves with the highest concentration selected and nanogel base as a negative control, then covered using gauze. Visual observations were made to see the presence or absence of erythema and edema [7].

Results and discussion

Results of macroscopic examination of breadfruit leaves (*artocarpus altilis*)

The results of macroscopic examination of the intact form of breadfruit leaves show leaves with pointed leaf bases, rough upper and lower surfaces, pinnate leaf veins, and leaf vein branches and leaf veins protruding on the lower surface. The results of this observation are in accordance with those listed in the Indonesian Herbal Pharmacopoeia [10].

Results of microscopic examination of simple drugs

The results of microscopic examination of breadfruit leaf simplisia powder showed the presence of stomata, calcium oxalate crystals, secretory glands, covering hairs and systolites. The results of this observation are in accordance with the Indonesian Herbal Pharmacopoeia. (**Figure 1**) [10].

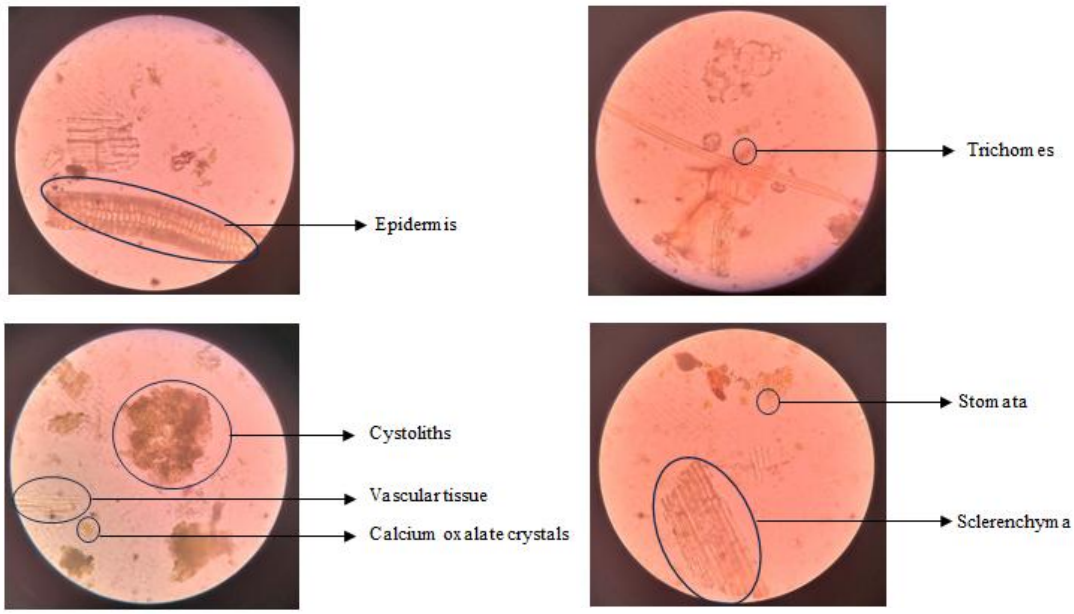


Figure 1 Microscopic view of breadfruit leaves.

Results of ethanol extract of breadfruit leaves

From 2,800 g of dry breadfruit leaf simplicia, 305 g of thick extract was produced, resulting in an extract yield of 10.89 % w/w. The results obtained met the requirements of herbal pharmacopoeia, namely not less than 9.9 % [10].

Results of antibacterial activity test of ethanol extract of breadfruit leaves

The results of the antibacterial activity test of ethanol extract of breadfruit leaves on *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria showed that the largest inhibition zone was at a concentration of 25 %, which can be seen in **Tables 2**.

Table 2 Results of antibacterial activity tests of ethanol extract of breadfruit leaves against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria.

Concentrations (%)	**Diameter of the growth inhibition zone of bacteria (mm) X ± SD**	
	Staphylococcus aureus	***Pseudomonas aeruginosa* bacteria**
	X ± SD	**X ± SD**
1.5625	12.5 ± 1.03	10.83 ± 0.30
3.125	12.86 ± 1.10	11.1 ± 0.4
6.25	13.1 ± 1.04	11.46 ± 0.25
12.5	14.83 ± 2.33	12.3 ± 0.81
25	13.66 ± 0.55	12.86 ± 0.66
50	13.16 ± 0.30	11.6 ± 0.36
100	12.33 ± 0.25	9.83 ± 0.25
Positive Control (Bioplacenton®)	26.3 ± 0.17	26.86 ± 0.05

The results of the antibacterial activity test of ethanol extract of breadfruit leaves in **Table 2** which has the largest diameter to inhibit the growth of Staphylococcus aureus and Pseudomonas aeruginosa bacteria at 25 % extract concentration with a diameter of 13.66 and 12.86 mm. However, at concentrations higher than 25 %, the inhibition zone value is getting smaller. The increase in extract concentration above 25 % did not increase the zone of inhibition. The increase in concentration may not be directly proportional to the diameter of the inhibition zone, this can occur because high concentrations make the solubility of the extract more concentrated so that the extract is less perfectly diffused into the agar [15]. However, in making the breadfruit leaf extract nanogel formulation in this study, the concentrations used were 1.5625, 3.125 and 6.25 % because the 3 concentrations had produced relatively strong inhibition, in addition because the preparation to be made was a nanogel with the ability to increase penetration into the skin better so as to reduce the concentration of active substances so as to reduce the risk of toxicity.

Results of nanogel preparation formulation

Carbopol 940 was used as a gel base with a concentration of 0.5 % because it produced a thick nanogel with a distinctive and stable aroma compared to a Carbopol 940 concentration of 1 % which produced a very thick nanogel preparation that did not flow.

Measurement of nanogel preparation particles

Particle size measurements were conducted at the Nanomedicine Laboratory, Faculty of Pharmacy, University of Sumatera Utara. The average results of nanogel particle size measurements and graphs of changes in nanogel particle size due to storage time can be seen in **Table 3**.

Table 3 Nanogel article size test result data.

Storage (weeks)	Nanogel			
	F0	**F1**	**F2**	**F3**
0	102.02	112.77	100.23	178.44
6	169.13	133.86	179.07	283.88
12	235.91	234.00	247.12	334.85

Descriptions; a = (F0 = nanogel without extract), b = (F1 = nanogel ethanol extract of breadfruit leaves (1.5625 %), F2 = nanogel ethanol extract of breadfruit leaves (3.125 %), F3 = nanogel ethanol extract of breadfruit leaves (6.25 %)).

Table 3 shows the particle size results of breadfruit leaf ethanol extract nanogels stored for 12 weeks. All formulated breadfruit leaf ethanol extract nanogels experienced an increase in particle size. The increase in particle size that occurred was still within the range of particle size requirements, which did not exceed 1,000 nm from the beginning of the formulation until 12 weeks of storage. This increase occurred due to a decrease in the effectiveness of surfactants so as to reduce the interfacial tension due to increased absorption and due to changes in temperature during testing. Therefore, in order to keep the particle size stable during storage, the particle preparation is stored at room temperature or in a dry place and not exposed to sunlight [16].

Organoleptic and homogeneity test results

The results of the organoleptic and homogeneity tests can be seen in **Table 4** and **Figure 2**.

Table 4 Organoleptic test results.

Formula	Concentrations	Organoleptic		
		Color	**Odor**	**Consistency**
F0	Basis nanogel	Clear	Odorless	Thick
F1	1.5625 %	Light Green	Distinctively	Thick
F2	3.125 %	Green	Distinctively	Thick
F3	6.25 %	Dark Brown	Distinctively	Thick

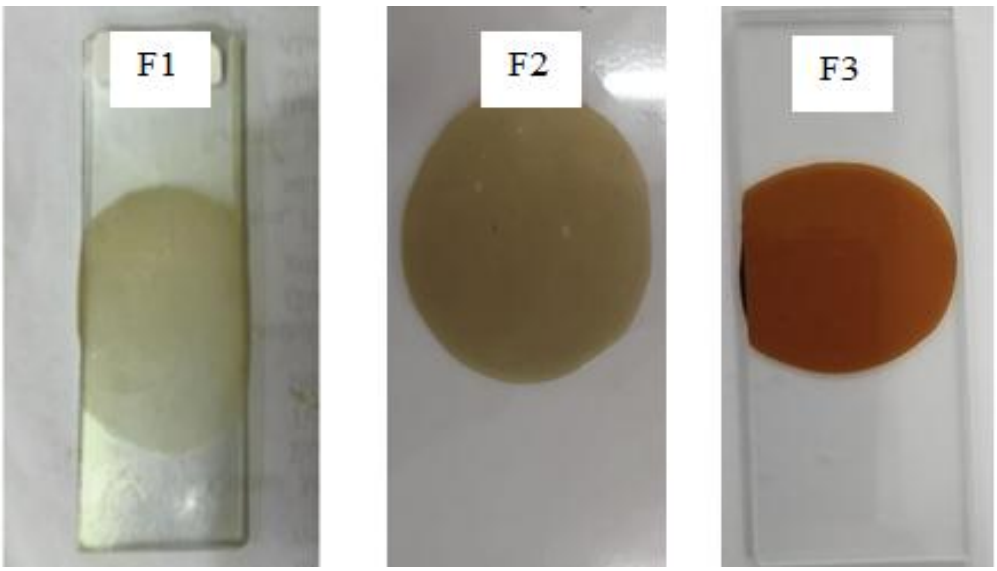


Figure 2 Homogeneity of preparation.

The results of homogeneity observations show that the 3 formulas have good homogeneity, showing no particles on the object glass. The preparation is said to be homogeneous when there is an even color and no different particles are found.

Spread power check

The resulting spreadability shows good nanogel preparation which can be seen in **Table 5**.

Table 5 results of evaluation of spreading power of nanogel preparation with additional load.

Additional load (g)	Spread power diameter results (cm) (average ± SD)			
	F0	F1	F2	F3
0	5.0 ± 0.0	5.0 ± 0.0	5.1 ± 0.0	5.0 ± 0.0
50	5.2 ± 0.1	5.1 ± 0.1	5.2 ± 0.05	5.3 ± 0.0
100	5.7 ± 0.1	5.5 ± 0.1	5.5 ± 0.05	5.4 ± 0.05
125	6.1 ± 0.1	6.0 ± 0.05	5.9 ± 0.05	6.1 ± 0.1

The results of the examination of the spreadability of the nanogel preparation show that the spreadability of the nanogel preparation increases with increasing load and is still within the range of the requirements for the spreadability of semi-solid preparations, namely 5 - 7 cm. The results of the examination of the spreadability of the nanogel preparation show that the spreadability of the nanogel preparation increases with increasing load and is still within the range of the requirements for the spreadability of semi-solid preparation.

Transmittance test results

The transmittance test results can be seen in **Table 6** as follows.

Table 6 Transmittance test results of nanogel preparations.

Formula	Transmittance (%)
F1 (1.5625 %)	92.17
F2 (3.125 %)	92.81
F3 (6.25 %)	92.98

All formulas fulfilled the transmittance test requirements with a percentage above 90 %. The transmittance percentage requirement is 90 - 100 %, with the results obtained being the formation of nanogels that are able to produce small droplet sizes. The higher the transmittance percentage value indicates that the nanogel droplet size formed is finer [17].

Storage stability

Storage stability can be seen in **Table 7** below.

Table 7 The effect of low temperature, room temperature and high temperature on the stability of nanogel preparations.

Storage period (weeks)	Organoleptic											
	Color			Odor			Consistency			Phase separation		
	F1	F2	F3	F1	F2	F3	F1	F2	F3	F1	F2	F3
0	LG	G	DB	D	D	D	Th	Th	Th	N	N	N
1	LG	G	DB	D	D	D	Th	Th	Th	N	N	N
2	LG	G	DB	D	D	D	Th	Th	Th	N	N	N
3	LG	G	DB	D	D	D	Th	Th	Th	N	N	N

Storage period (weeks)	Organoleptic											
	Color			Odor			Consistency			Phase separation		
	F1	F2	F3	F1	F2	F3	F1	F2	F3	F1	F2	F3
4	LG	G	DB	D	D	D	Th	Th	Th	N	N	N
5	LG	G	DB	D	D	D	Th	Th	Th	N	N	N
6	LG	G	DB	D	D	D	Th	Th	Th	N	N	N
7	LG	G	DB	D	D	D	Th	Th	Th	N	N	N
8	LG	G	DB	D	D	D	Th	Th	Th	N	N	N
9	LG	G	DB	D	D	D	Th	Th	Th	N	N	N
10	LG	G	DB	D	D	D	Th	Th	Th	N	N	N
11	LG	G	DB	D	D	D	Th	Th	Th	N	N	N
12	LG	G	DB	D	D	D	Th	Th	Th	N	N	N

Descriptions; F1= nanogel ethanol extract of breadfruit leaves 1.5625 %, F2 = nanogel ethanol extract of breadfruit leaves 3.125 %, F3 = nanogel ethanol extract of breadfruit leaves 6.25 %, LG = Light Green, H = Green, DB = Dark Brown, D = Distinctively, Th = Thick, N = No phase separation.

Based on **Table 7** shows that the color, odor and consistency of the nanogel preparation stored at low temperature, room temperature and high temperature did not change from the beginning of observation to storage for 12 weeks. This shows that the nanogel and gel preparations from breadfruit leaf extract are stable.

pH test

The results of the pH test can be seen in **Table 8** below.

Table 8 Nanogel pH measurement data on storage for 12 weeks.

Storage period (weeks)	Average pH ± SD											
	Low temperature (4 °C)				Room temperature (25 °C)				High temperature (40 °C)			
	F0	F1	F2	F3	F0	F1	F2	F3	F0	F1	F2	F3
0	4.96 ± 0.02	5.37 ± 0.01	5.46 ± 0.005	5.97 ± 0.04	4.95 ± 0.005	5.33 ± 0.011	5.44 ± 0.015	5.94 ± 0.011	4.92 ± 0.005	5.30 ± 0.01	5.41 ± 0.01	5.89 ± 0.005
2	4.95 ± 0.005	5.36 ± 0.005	5.46 ± 0.011	5.90 ± 0.005	4.94 ± 0.005	5.31 ± 0.005	5.43 ± 0.005	5.88 ± 0.015	4.90 ± 0.01	5.28 ± 0.005	5.39 ± 0.005	5.87 ± 0.011
4	4.94 ± 0.005	5.33 ± 0.015	5.46 ± 0.005	5.86 ± 0.005	4.92 ± 0.015	5.3 ± 0.000	5.41 ± 0.01	5.84 ± 0.005	4.89 ± 0.01	5.27 ± 0.005	5.37 ± 0.011	5.84 ± 0.000
6	4.92 ± 0.01	5.32 ± 0.005	5.45 ± 0.01	5.81 ± 0.017	4.91 ± 0.011	5.29 ± 0.01	5.39 ± 0.005	5.81 ± 0.011	4.85 ± 0.017	5.25 ± 0.005	5.37 ± 0.000	5.80 ± 0.005
8	4.89 ± 0.011	5.30 ± 0.005	5.43 ± 0.005	5.77 ± 0.015	4.88 ± 0.01	5.26 ± 0.01	5.36 ± 0.026	5.75 ± 0.011	4.81 ± 0.01	5.22 ± 0.011	5.34 ± 0.011	5.73 ± 0.017
10	4.85 ± 0.00	5.28 ± 0.01	5.42 ± 0.011	5.74 ± 0.005	4.84 ± 0.005	5.23 ± 0.011	5.35 ± 0.01	5.71 ± 0.005	4.79 ± 0.01	5.20 ± 0.01	5.32 ± 0.015	5.68 ± 0.011
12	4.81 ± 0.02	5.22 ± 0.025	5.40 ± 0.015	5.71 ± 0.005	4.80 ± 0.011	5.21 ± 0.015	5.32 ± 0.011	5.66 ± 0.015	4.74 ± 0.015	5.17 ± 0.01	5.31 ± 0.000	5.64 ± 0.011

Based on **Table 8**, there is a decrease in pH during storage, but the decrease in pH is relatively stable because it is still in accordance with the pH of the skin, which is 4.5 - 6.5 so it is safe to use. If the pH of the preparation is lower than the pH of the skin, it can cause irritation to the skin, but if the pH of the preparation is higher than the pH of the skin, it will cause scaly skin [16].

Viscosity test results

The results of the viscosity measurement of the preparation can be seen in **Table 9** below.

Table 9 Viscosity measurement data of nanogels after 12 weeks of storage at room temperature.

Storage period (weeks)	Viscosity ± SD			
	F0	**F1**	**F2**	**F3**
0	2,970 ± 36.05	3,100 ± 10	3,453 ± 40.10	3,671 ± 23.62
2	2,852 ± 37.5	3,031 ± 41.93	3,370 ± 8.50	3,528 ± 19.67
4	2,776 ± 15.27	2,924 ± 14.01	3,287 ± 15.53	3,452 ± 10.21
6	2,678 ± 10.40	2,835 ± 37.74	3,208 ± 10.96	3,392 ± 7.09
8	2,584 ± 55.19	2,733 ± 20.81	3,178 ± 6	3,342 ± 7.50
10	2,481 ± 8.54	2,682 ± 6.80	3,119 ± 19	3,286 ± 15.71
12	2,390 ± 11.23	2,533 ± 41.07	3,067 ± 16.62	3,227 ± 15.71

Based on the results of the viscosity test, it can be concluded that along with the length of storage there is a decrease in the viscosity of the nanogel preparation. Therefore, it can be concluded that the longer the storage, the viscosity of a preparation will decrease. This decrease in viscosity occurs due to the alkaline nature of the gel base used, namely carbopol 940, where the hygroscopic nature of carbopol is able to absorb water vapour which causes the viscosity of the nanogel to decrease [17]. However, the viscosity value of the gel is still in the specified range of 2,000 - 4,000 cPs [18].

Centrifugation test results

This test was carried out at the beginning after the preparation was made with a measurement of 1 time. The preparation was put into a centrifuge tube and then centrifuged at a speed of 5,000 rpm for 30 min. The test results can be seen in **Table 10** and **Figure 3**.

Table 10 Centrifugation test results.

Formula	Centrifugation
	Phase separation
F1	-
F2	-
F3	-

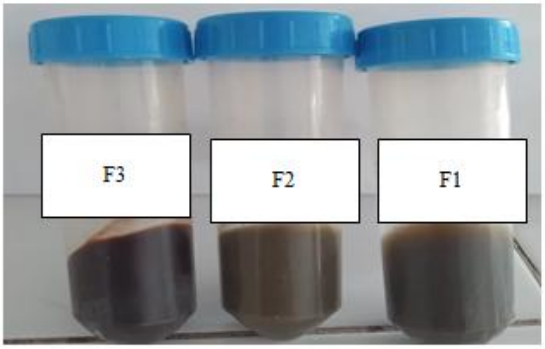


Figure 3 Centrifugation test results.

Cycling test results

Stability testing with cycling test was carried out at extreme temperatures alternately, namely hot temperature of 40 °C and cold temperature of 4 °C each for 24 h. This treatment is called 1 cycle, and this test was carried out for 6 cycles (12 days) [18]. After testing the preparation showed no changes in shape, odor and color in the 3 preparation formulas. So this preparation is declared stable during the cycling test.

Results of antibacterial activity tests of breadfruit leaf ethanol extract nanogels

The results of the antibacterial activity test of breadfruit leaf ethanol extract nanogel can be seen in **Table 11**.

Table 11 Results of the inhibition zone diameter of the breadfruit leaf ethanol extract nanogel preparation.

Bacteria	Formula				
	K+	F0	F1	F2	F3
Staphylococcus aureus	20.6 ± 0.6	0	9.4 ± 0.2	9.7 ± 0.2	10.1 ± 0.1
Pseudomonas aeruginosa	23.9 ± 0.4	0	9.9 ± 0.1	10.3 ± 0.2	11 ± 0.2

Descriptions: K+ = Bioplacenton ®, F0 = nanogel without extract (Base), F1 = nanogel ethanol extract of breadfruit leaves 1.5625 %, F2 = nanogel ethanol extract of breadfruit leaves 3.125 %, F3 = nanogel ethanol extract of breadfruit leaves 6.25 %.

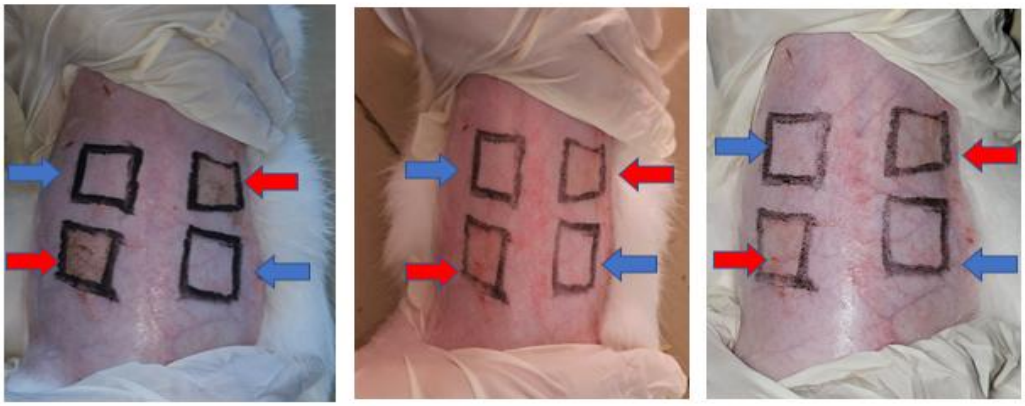
The results of the examination of antibacterial activity of nanogel preparations of ethanol extract of breadfruit leaves F1, F2 and F3 have antibacterial activity indicated by the presence of inhibition zone formed around the disc paper. The diameter of the inhibition zone of nanogel is smaller than the extract form. The decrease in the diameter of the inhibition zone can be caused because the base has difficulty diffusing properly which causes the active substance not to be released properly so that the inhibition decreases [19].

Results of irritation test on rabbit skin

Irritation tests were carried out on nanogel preparations with the highest extract concentration, namely 6.25 %, to determine the presence of allergic/irritation reactions from the preparations made, which can be seen in **Table 12** and **Figure 4**.

Table 12 Irritation test data of the preparation on rabbit skin.

Observation time (h)	Irritation effect	Test group (Score)	
		F0 (Base)	F3 (6.25 %)
24	Erythema	0	0
	Edema	0	0
48	Erythema	0	0
	Edema	0	0
72	Erythema	0	0
	Edema	0	0



Note: Nanogel exposure location Ethanol extract of breadfruit leaves 6.25 %.
Nanogel exposure location without extract.

Figure 4 Irritation test results of nanogel preparations.

The results of the irritation test of the ethanol extract nanogel preparation of breadfruit leaves with a concentration of 6.25 % did not cause irritation, this is because the pH of the preparation is still in accordance with the pH of the skin, with the Primary Irritation Index (PII) equal to 0, so the study was continued with testing the activity of healing burns.

Results of burn wound healing effectiveness test

In this study, the animals used were 25 male rats that were burned, the animals were given drugs or substances according to the treatment group for up to 21 days and the healing rate was observed. After 21 days, the rats were given treatment by taking skin sections for microscopic examination. Data on wound diameter in the positive control group (bioplacenton®), F0 (nanogel without extract (Base)), F1 (1.5625 %), F2 (3.125 %), F3 (6.25 %) can be seen in **Table 13**.

Table 13 Percentage of burn healing.

Days	Percentage of burn healing				
	K- (F0)	K+	F1	F2	F3
3	9.55 ± 6.06	23.62 ± 4.45	12.01 ± 6.33	12.16 ± 2.79	15.13 ± 1.46
6	15.24 ± 7.7	26.17 ± 5.78	17.48 ± 8.12	16.21 ± 4.44	19.13 ± 2.49
9	23.05 ± 7.72[a]	39.22 ± 3.11[b]	25.55 ± 10.11[b]	32.57 ± 3.31[b]	31.34 ± 3.27[b]
12	43.80 ± 11.61	60.61 ± 7.78	50.77 ± 15.69	60.42 ± 6.11	48.09 ± 9.46
15	49.82 ± 12.68	64.71 ± 9.04	56.96 ± 18.49	71.04 ± 16.73	67.35 ± 8.43
18	53.29 ± 12.67[a]	74.70 ± 12.79[b]	73.97 ± 17.62[b]	81.19 ± 12.95	79.79 ± 12.01[b]
21	56,58 ± 12,62[a]	93.11 ± 13.77[b]	81.14 ± 21.11[b]	92.84 ± 14.30[b]	88.91 ± 10.21[b]

Descriptions: K+ = Bioplacenton ®, F0 = (nanogel without extract (Base)), F1 = nanogel ethanol extract of breadfruit leaves 1.5625 %, F2 = nanogel ethanol extract of breadfruit leaves 3.125 %, F3 = nanogel ethanol extract of breadfruit leaves 6.25 %, a = there is a significant difference with the positive control ($p < 0.05$), b = there is a significant difference with the negative control ($p < 0.05$).

The nanogel group without extract (F0) above can be seen the average percentage of burn healing of 5 rats given nanogel preparations without extract showed a healing percentage of 56.56 % on day 21, the positive control group showed an average percentage of burn healing reached 93, 11 % on day 21, 1.5625 %

breadfruit leaf ethanol extract nanogel group (F1) average healing percentage reached 81.14 % on day 21, 3.125 % breadfruit leaf ethanol extract nanogel group (F2) healing percentage reached 92.85 and 6.25 % breadfruit leaf ethanol extract nanogel group (F3) healing percentage reached 88.91 %.

From the data above, it can be seen that the average burn wound healing occurs on day 21. In this study, the normality test used the Shapiro Wilk test, the computational results showed that some data were not normally distributed where $p < 0.05$, so the alternative test was the Kruskal Wallis test. The results of the Kruskal Wallis test showed that there was a p value < 0.05, which means that there are differences in healing days in the 5 groups. Because the results of the Kruskal Wallis test were different, further testing was carried out to see which groups were different. so that a further test (Post Hoc Test) was carried out, namely the Mann Whitney test. The computational results of the Mann Whitney test showed that the healing time of the blank group (formulation without extract) with Bioplacenton was different with a p value < 0.05 where the positive control group healed faster than the formula without extract. In the formula group without extract and nanogel of ethanol extract of breadfruit leaves, there was a difference with a value of $p < 0.05$, where the nanogel group of ethanol extract of breadfruit leaves healed faster than the formula without extract. The average healing time of the positive control group was not significantly different from the 1.565 and 3.125 % breadfruit leaf ethanol extract nanogel groups, but the 3.125 % breadfruit leaf ethanol extract nanogel showed a healing percentage close to the positive control.

These results are not in line with research conducted by Kurniawan who made a gel preparation with ethanol extract of breadfruit leaves to accelerate burn healing where the concentration of 6.25 % had a faster healing period of 19 days [9], in contrast to this study where the preparation of breadfruit leaf ethanol extract nanogel with a concentration of 3.125 % showed better healing than the concentration of 6.25 % within 21 days. Gel and nanogel preparations of ethanol extract of breadfruit leaves have a time difference that is not much different in healing burn wounds. The difference can occur due to the different places where breadfruit leaves are taken. Some environmental factors that can influence are environmental conditions such as temperature, humidity, light and air quality in the place of growth which play a role in influencing metabolism and other biological processes.

Histology test results

Angiogenesis

In this observation, the number of blood vessels formed will be counted and then observed using a microscope with a magnification of 10×40 in 3 fields of view and compared between groups as can be seen in **Table 14** and **Figure 5**.

Table 14 Average results of angiogenesis evaluation of each group.

Groups	Average angiogenesis ± SD
Positive control (Bioplacenton®)	14.4 ± 2.9
F0 (Nanogel without extract)	7 ± 1.5
F1 (nanogel ethanol extract of breadfruit leaves 1.5625 %)	9.4 ±2.9
F2 (nanogel ethanol extract of breadfruit leaves 3.125 %	14 ± 3.3
F3 (nanogel ethanol extract of breadfruit leaves 6.25 %)	10 ± 2.4

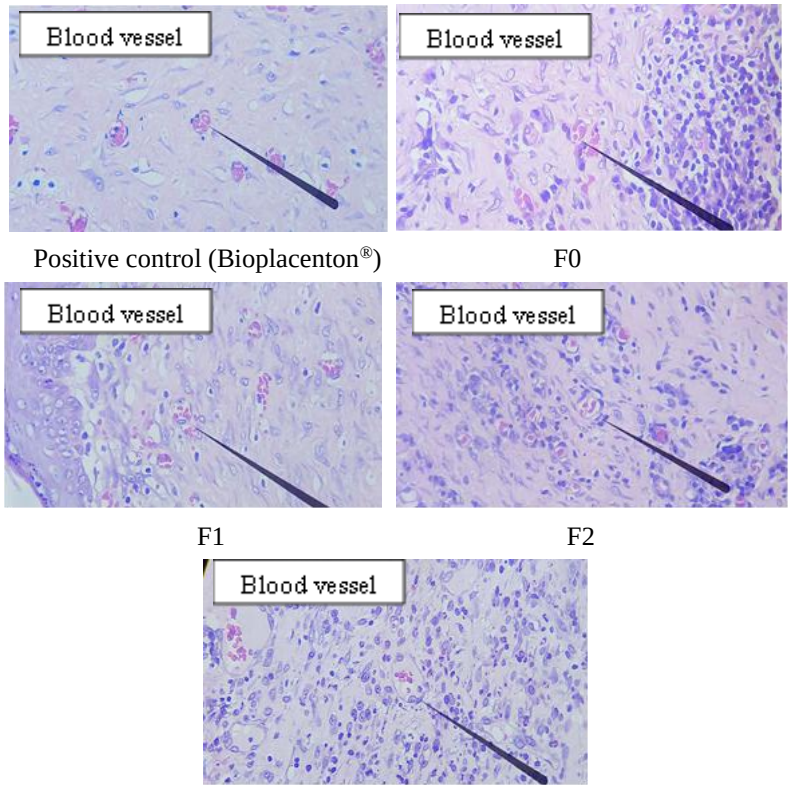


Figure 5 Angiogenesis in rat skin tissue.

In statistical analysis, the normality test used is Shapiro-Wilk where the data obtained is normally distributed and homogeneous with a *p* value > 0.05. Based on the results of the One Way ANOVA test, the *p* value was 0.006. Therefore, it can be concluded that there is a difference between the control group and the formula. Because the results of the One Way ANOVA test showed a significant difference, further tests were carried out to see which groups were different so that a further test (Post Hoc Test) used was Bonferroni. The results of the Bonferroni test showed that the positive control group was not significantly different from the group with 3.125 % Nanogel formula. The negative control group was not significantly different from the groups with Nanogel formulas of 1.5625 and 6.25 %.

In wounds that are in the process of healing, new capillaries grow into the wound and produce new blood vessels similar to normal skin. Secondary metabolites that play a role in the angiogenesis process are tannins and terpenoids. Tannins modulate growth factors such as VEGF (Vascular Endothelial Growth Factor) that are important in the formation of new blood vessels. Terpenoids activate angiogenic pathways such as limonene which can stimulate signalling pathways associated with angiogenesis [20].

Fibroblast

In this observation, the number of fibroblasts formed will be calculated and then observed using a microscope with a magnification of 10×40 in 3 fields of view and compared between groups as can be seen in **Table 15** and **Figure 6**.

Table 15 Average results of fibroblast evaluation of each group.

Groups	Average Fibroblast ± SD
Positive control (Bioplacenton®)	148 ± 15
F0 (Nanogel without extract)	50 ± 14
F1 (nanogel ethanol extract of breadfruit leaves 1.5625 %)	95 ± 10

Groups	Average Fibroblast ± SD
F2 (nanogel ethanol extract of breadfruit leaves 3.125 %	148 ± 16
F3 (nanogel ethanol extract of breadfruit leaves 6.25 %)	132 ± 12

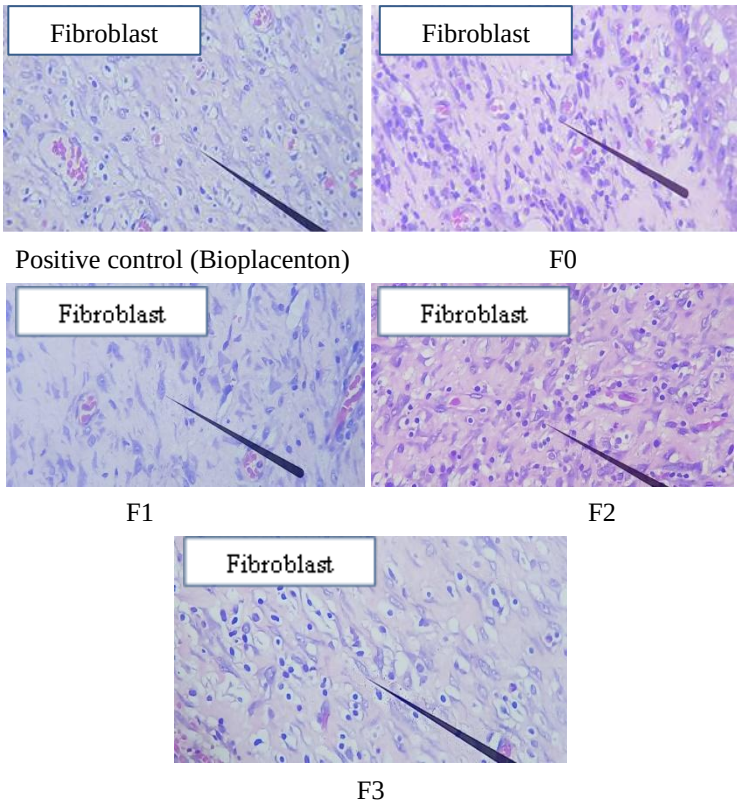


Figure 6 Fibroblast cells in rat skin tissue.

Based on the One Way ANOVA test results, the *p* value is 0.000. Therefore, it can be concluded that there is a difference between the control group and the formula. Because the results of the One Way ANOVA test showed significant differences, further tests were carried out to see which groups were different so that the Post Hoc Test used was Bonferroni. The results of the Bonferroni test showed that the positive control group was not significantly different from the group with the nanogel formula of 3.125 % ethanol extract and 6.25 % breadfruit leaves. The negative control group was significantly different from the group with the formula with the extract.

The role of fibroblasts in wound healing consists of the homeostasis, inflammation, proliferation and maturation phases. Fibroblast cells are the most common cells found in connective tissue and synthesize several extracellular matrix components such as collagen, elastin and reticular. Fibroblasts will produce collagen which will affect the reepithelialization process that will close the wound. Secondary metabolites that play a role in the formation of fibroblast cells are alkaloids by modulating the cell cycle or stimulating the activation of certain signal pathways that regulate the growth of fibroblast cells. Alkaloids have anti-inflammatory properties that can help fibroblast cells function better in repairing tissue after injury [21].

Collagen

The density of collagen fibers formed in the wound healing process was calculated after being stained with HE and observed using a microscope which can be seen in **Table 16** and **Figure 7** with a magnification of 10x throughout the field of view. The collagen score can be seen in the description below:

0 = no collagen fibers found

1 = low density of collagen fibers in the wound area (< 10 %)

2 = moderate density of collagen fibers in the wound area (10 - 50 %)
3 = dense density of collagen fibers in the wound area (50 - 90 %)
4 = very dense density of collagen fibers in the wound area (90 - 100 %)

Table 16 Collagen fiber density scoring results.

Treatment groups	Collagen density scoring
Positive control (Bioplacenton®)	4
F0 (Nanogel without extract)	1
F1 (nanogel ethanol extract of breadfruit leaves 1.5625 %)	1
F2 (nanogel ethanol extract of breadfruit leaves 3.125 %	3
F3 (nanogel ethanol extract of breadfruit leaves 6.25 %)	3

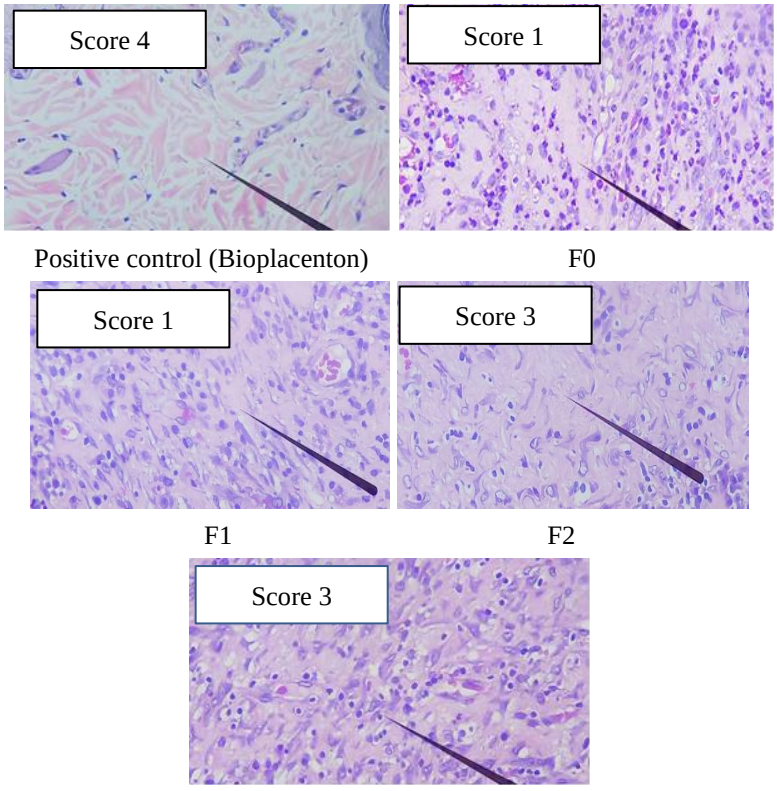


Figure 7 Collagen in rat skin tissue.

In statistical analysis, the normality test used was Shapiro-Wilk. The data was declared not normally distributed with a *p* value of 0.05, so the test used was Kruskal-Wallis. Based on the Kruskal Wallis test, there was a significant difference in the average collagen score in wound healing. The results showed that the positive control group and the breadfruit leaf ethanol extract nanogel group experienced an increase in the number of collagen fibers, this was because the group had been applied with active ingredients that played a role in the wound healing process. The positive control group and the breadfruit leaf ethanol extract nanogel group 3.125 % (F2) and 6.25 % (F3) experienced the highest increase when compared to the group with an extract concentration of 1.5625 %. Furthermore, a further Mann Whitney test was carried out, the results of the breadfruit leaf ethanol extract nanogel 1.5625, 3.125, and 6.25 % were significantly different from the formula group without extract.

The secondary metabolite content that plays a role in collagen formation is saponin by activating enzymes involved in collagen biosynthesis such as prolyl hydroxylase which is important for the formation of stable and well-functioning collagen fibers [21].

Research limitations

The limitations in this study are the lack of testing on several parameters for nanometer-scale preparations, such as zeta potential testing to determine the stability of the dispersion of nanogel preparations, Transmission Electron Microscope to determine the shape and surface structure of nanogel particles.

Conclusions

Ethanol extract of breadfruit leaves and nanogel preparations of ethanol extract of breadfruit leaves have antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa bacteria which often infect human skin, especially open wounds. Nanogel of ethanol extract of breadfruit leaves with a concentration of 3.125 % (F2) provided a better healing effect on burns in mice with an average percentage of burn healing of 92.84 % on day 21, while a concentration of 6.25 % (F3) with an average percentage of burn healing of 88.91 % on day 21, and a concentration of 1.5625 % (F1) with an average percentage of burn healing of 81.14 % on day 21.

Future directions

It is recommended to conduct zeta potential value measurements, Transmission Electron Microscope (TEM) and clinical trials on humans to validate the efficacy of the ethanol extract nanogel formulation of breadfruit leaves in healing burns.

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References

[1] Apriliani, Fathonah and Ashari. Anti-inflammatory effectiveness test of 70 % ethanol extract of dewa leaves (gynur pseudochina (L.) Dc.) on burns of male white rats of wistar strain. muhammadiyah college of pharmacy tangerang. *Farmagazine Journal* 2021; **8(2)**, 53-55.

[2] W Antoro and IM Sari. Overview of the level of mother's knowledge about first aid for burns in toddlers. *Multidisciplinary Scientific Journal* 2022; **1(2)**, 4452.

[3] Suriawanto, Setyawati and Narwan. The effect of giving stingless bee propolis extract on the healing of burns in white rats (rattus norvegicus). widya nusantara palu college of health sciences. *Indonesian Journal of Biotechnology and Bioscience* 2021; **8(1)**, 69-70.

[4] Herdini, S Bahri and Y Natasya. Antibacterial activity test of ethanol extract of tinospora cordifolia (willd.) miers leaves against staphylococcus aureus and pseudomonas aeruginosa bacteria. *Sainstech Journal* 2024; **34(2)**, 32.

[5] Mahdani, Rizal and Amiryah. Evaluation of infection incidence in burn patients hospitalized at dr. zainoel abidin regional hospital. *Journal of Medical Science* 2022; **3(2),** 71-79.

[6] R Rizkyana, Sukarsa, P Widodo and D Palupi. Morphological diversity of breadfruit (*artocarpus altilis* (park.) fosberg. var. non-seminiferus) in banyumas area. *Scientific Journal of Biology Unsoed* 2022; **4(3)**, 167-169.

[7] SU Karo-Karo. 2023, Formulation of nanogel preparations containing ethanol extract of papaya leaves (carica papaya l.) and test of burn wound healing activity in rats. Master Thesis. Universitas Sumatera Utara, North Sumatra, Indonesia.

[8] ND Harahap. 2023, Effectiveness test of combination gel preparation of ethanol extract of tekelan leaves (chromolaena odorata l.) and ethanol extract of petai cina leaves (leucaena leucocephala l.) on healing of infected cut wounds in white rats (rattus norvegicus L.). Master Thesis. Universitas Sumatera Utara, North Sumatra, Indonesia.

[9] Y Kurniawan. Administration of breadfruit leaf extract gel (*Artocarpus altilis*) can accelerate the healing process of burns in mice. *Syifa Medika Journal* 2019; **8(1)**, 30-35.

[10] Ministry of Health of the Republic of Indonesia. *Indonesian herbal pharmacopoeia*. Ministry of Health of the Republic of Indonesia. Jakarta, Indonesia, 2017.

[11] SE Priani, SY Somantri and R Aryani. Formulation and Characterization of SNEDDS (self nanoemulsifying drug delivery system) containing black cumin oil and olive oil. *Journal of Pharmaceutical and Clinical Sciences* 2020; **7(1)**, 33.

[12] A Indalifiany, MH Malaka, Sahidin, A Fristiohady and R Andriani. Formulation and physical stability test of nanoemulgel containing petrosia Sp. Ethanolic extract. *Jurnal Farmasi Sains dan Praktis* 2021; **7(3)**, 321-331.

[13] Y Nailufa and YA Najih. Epigallocatechin gallate cream formulation as anti aging. *Journal of Pharmacy and Science* 2020; **5(2)**, 82-83.

[14] LG Zulkarya and ED Hastuti. Formulation of brown seaweed (*padina australis*) ethanol extract cream preparation and antioxidant activity test using DPPH. *Cendekia Journal of Pharmacy* 2018; **2(1)**, 85.

[15] M Jufri, R Iswandana, DA Wardani and SF Malik. Formulation of red fruit oil nanoemulsion using sucrose palmitate. *International Journal of Applied Pharmaceutics*. 2022; **14(5)**, 175-180.

[16] APR Nurhamidin, Fatimaali and I Anastasia. Antibacterial activity test of n-hexane extract of langsat fruit SEEDS (Lansium domesticum Corr) against *Staphylococcus aureus* and *Klebsiella Pneumoniae* Bacteria. *Pharmacon* 2021; **10(1)**, 748-755.

[17] EP Sari, U Lestari and Syamsurizal. Physicochemical properties test of fractionated lotion of dichloromethane extract of *artocarpus altilis* fruit peel. *Scientific Journal of Applied Sciences* 2021; **5(2)**, 131.

[18] L Ariani and Wulandari. *Physical stability of olive oil nanogel (olea europaeae L.)*. Jurnal Ilmiah Cendekia Eksakta. Semarang, Indonesia, 2020.

[19] Purwanti and Verryanti. Antioxidant activity and physical evaluation of peel-off gel mask preparations from purple eggplant skin extract (salonum melongena I.) *Indoensi Natural Research Pharmaceutical Journal* 2016; **1(2)**, 10-21.

[20] D Febriyanti, E Halimatushadyah, DA Waluyo and K Rahma. Formulation and test of anti-acne cream from starfruit leaf extract (Averrhoa bilimbi L.) against Staphylococcus epidermis bacteria. *CERATA Journal of Pharmaceutical Sciences* 2023; **14(2)**, 137.

[21] Maharani and MD Farabi. Phytochemical test of dried breadfruit leaf extract *(Artocarpus altilis). In*: Proceedings of the 12th International Conference On Lesson Study (ICLS-XII). Java, Indonesia. 2021.