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Test of the activity of 96% ethanol extract gel of avocado seeds (Persea americana mill.) on burn wound healing in the back of new zealand rabbits

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ABSTRACT

Avocado seeds contain secondary metabolite compounds in the form of alkaloids, flavonoids, saponins, tannins, triterpenoids and steroids. The mechanism of flavonoids in inhibiting the inflammatory process in burns is through various methods, namely inhibiting capillary permeability, inhibiting the release of serotonin and histamine to the site of inflammation. In this study, avocado seed extract was obtained by maceration method and formulated into a gel preparation with a concentration of 5%, 10% and 15%. The gel base was used as a negative control, and the gel containing 10% placenta extract and 0.5% neomycin sulfate was used as a positive control, applied to burn wounds on the backs of New Zealand rabbits with a diameter of 2 cm. The activity test of avocado seed extract gel was conducted on 3 rabbits, with each formulation applied 3 times, and the wound diameter was measured daily. This test was conducted for ten days. Avocado seeds (Persea americana Mill.) contain alkaloids, flavonoids, tannins, saponins and titerpenoids. The results of observations of the healing and drying process of burns, avocado seed extract gel (Persea americana Mill.) on the 10th day were F1 (5%) wound diameter 0.2 cm, F2 (10%) wound diameter 0.1 cm, F3 (15%) wound diameter 0 cm. Avocado seed extract gel (Persea americana Mill.) with a concentration of 15% can provide the best effect on healing burns.

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1. Introduction

Wounds are damage to the skin [1] that is important for the human body, which can be caused by ulcers, trauma, scratches, or sharp objects. Burns occur due to contact with heat sources such as fire, water, chemicals, electricity, or radiation, which can affect other body systems [2]. Burns can be divided into three phases: the acute phase, subacute phase, and advanced phase. The acute phase involves threats to airway and circulation, the subacute phase involves inflammation and infection, and the advanced phase focuses on healing and complications such as scarring [3]. Burns are classified into three degrees: degree I (damage only to the epidermis, mild pain), degree II (damage to the epidermis and dermis, with blisters and pain), and degree III (damage to the entire skin and tissue, with permanent damage) [4].

Avocado seeds contain compounds such as saponins, tannins, and flavonoids (quercetin) that act as natural antioxidants and can reduce inflammation in burns [5]. Avocado seed extract also contains alkaloids, flavonoids, saponins, tannins, triterpenoids, and steroids that are beneficial for burn healing. Flavonoids can reduce inflammation, saponins accelerate collagen formation, and alkaloids and tannins have antiseptic effects [6].

This study aims to test the 96% ethanol extract of avocado seeds (Persea americana Mill.) for burn healing in New Zealand rabbits using the maceration extraction method. The extract is then formulated into a gel to test its effectiveness in burn wound healing.

2. Method

2.1. Research place

This research was conducted at the Laboratory of the University of Setia Budi Surakarta. Determination was carried out at the Ministry of Health Hospital Sardjito, Jl. Health No. 1 Sekip Yogyakarta.

2.2. Tools used in this study include

Shaving tools, stainles plates, measuring cups (pyrex), ovens (memmert), trays, sieves, blenders (Phillips), rabbit cages, KLT plates, glass beaker (pyrex), maceration vessels, rotary evaporators (heidolphs), stirring rods (pyrex), waterbath (mememet), test tubes (herms).7101), scales (Ohaus PX22), porcelain cups (pyrex), filter paper (whatman), watch glass (normax), muffle furnance (Nabertherm), pH meter (eutech pH6+), viscometer rion VT 04.

2.3. The materials used in this research include

Avocado seed extract, 96% ethanol, gel containing 10% placenta extract and 0.5% neomycin sulfate, Carbopol 940, methylparaben, propylene glycol, TEA, distilled water, concentrated HCl, Mayer's solution, Wagner's solution, chloroform, acetone, Liberman Burchad, Mg powder, 10% FeCl3 solution, acetic acid, CH3COOH, H2. piperine, and concentrated sulfuric acid, 10% NaOH, 2N HCl, ethyl acetate, methanol, n-butanole, n-hexane, acetic acid, New Zaeland rabbit.

2.4. Methods of collection of materials

Avocado seeds are obtained from avocados originating from Sumber Jati tree Village, Grobogan District, Grobogan Regency.

2.5. Simplicia Drying

Avocado seeds (Persea americana Mill.) that have been collected are peeled and then washed and cleaned with water. Then cut into four parts, then the avocado seeds are boiled for 5 minutes at 70oC, after boiling the avocado seeds are cut into thin slices 3 mm thick. After that, it is dried in an oven at a temperature of 40oC for 24 hours. After drying, the particles are reduced by blending and then sieved using a 60 mesh sieve to obtain seed powder [7].

2.6. Making Simplicia Powder

Dry avocado seeds, crushed until smooth using a blender, then sifted with a 60 mesh sieve. The purpose of sieving is to make storage easier and during the extraction process the surface area of the particles will be wider so that maximum active substances can be extracted [8].

2.7. Simplicity Parameter Test

2.7.1. Specific

2.7.1.1. Organoleptic test

This is done through simplicia identity and organoleptic testing to determine the shape, color, smell and taste of simplicia [9].

2.7.2. Non Specific

2.7.2.1. Total Ash Content

A total of 1 gram of sample was weighed and placed into a previously incandescent and weighed silicate crucible. The sample was then slowly incandescent using a furnace until the charcoal was used up, then cooled in a desiccator and weighed until the weight was stable [10].

2.7.2.2. Drying Shrinkage

1 gram of sample was weighed and placed in a porcelain cup that had been heated at 105°C for 30 minutes and weighed, the sample was leveled. The cup was placed in an oven, heated at 100-105°C, then weighed and heated again until the weight was stable [10]

2.8. Preparation of Avocado seed extract

The maceration process is carried out in a ratio of 1:10, putting 600 g of avocado seed powder into a macerator with 6 L of 96% ethanol solvent, soaking it for 6 hours while stirring occasionally, leaving it for 18 hours then filtering it and separating it from the filtrate [2]. In the same way, the extracted dregs are re-macerated with 2 L of 96% ethanol, and so on until the third maceration consumes 6 L of solvent. Next, all the filtrates were thickened with a rotary evaporator at a temperature of 40°C [11].

2.9. Parameter Extraction Test

2.9.1. Specific

2.9.1.1. Organoleptic Test

Carried out through extract identity and organoleptic testing to determine the shape, color, odor, and taste of the extract [10].

2.9.1.2. Identification of Alkaloids, Flavonoids, Saponins, Tannins, and Triterpenoids

Identification of alkaloids is done by adding 10 mg of the extract to 10 ml of HCl and heating for 2 minutes while stirring continuously. After the mixture cools, it is filtered to obtain the filtrate, which is then mixed with 5 ml of HCl and Wagner's reagent (iodine and potassium iodide). A positive result is indicated by the formation of a brown precipitate. For flavonoids, 0.2 grams of dry extract are placed in a test tube and treated with a few drops of 10% NaOH solution. A color change indicates the presence of flavonoids, as they are phenolic compounds. To identify saponins, 5 ml of the test extract is added to a test tube, shaken vertically for 10 seconds, and left for 10 seconds. The formation of stable foam of 1-10 cm height for at least 10 minutes suggests the presence of saponins. When 1 drop of 2N HCl is added, the foam does not disappear. For tannins, 2 ml of the test extract is divided into two parts, with one part used as a blank and the other reacting with 10% FeCl3 solution. A dark blue or greenish-black color indicates the presence of tannins [10]. Triterpenoids are identified by evaporating 5 ml of filtrate to dryness and adding Lieberman-Burchard reagent (2 drops of acetic acid anhydride and 1 drop of H2SO4). A blue/red color formation indicates the presence of triterpenoid compounds [12].

2.9.1.3. Identification Alkaloids, Flavonoids, Saponins, Tannins, and Triterpenoids of Thin Layer Chromatography

The identification of alkaloids is carried out using the mobile phase of ethyl acetate, methanol, and water (100:13.5:10), with piperine as the reference standard and Dragendorff reagent, where a positive reaction is indicated by blue or yellow fluorescence. For flavonoids, the mobile phase of n-butanol:acetic acid:water (4:1:5) is used with quercetin as the standard, and observations are made under UV light, where a positive result shows dark purple color or light blue fluorescence [13]. Saponin identification is conducted by adding 2M HCl to the sample, heating, neutralizing, evaporating, filtering, and then testing on a silica gel G60 plate using a chloroform:acetone mixture and spraying with SbCl3, with sapogenin as the standard [14]. For tannins, the mobile phase of methanol:water (6:4) is used with 5% FeCl3 solution and catechin as the standard, resulting in a black stain as the positive reaction [13]. Triterpenoid identification uses the mobile phase of n-hexane:ethyl acetate (5:5) with β-sitosterol as the standard, resulting in a blue stain under UV 254 nm light and a black color after spraying with Liebermann-Burchard reagent [14].

2.9.2. Specific

2.9.2.1. Drying Loss

1 gram of extract was weighed in a cup that had been heated at 105°C for 30 minutes and weighed. The extract was spread out to form a layer 5-10 mm thick. Then dried at a constant temperature until the weight was stable. After that, the cup was opened and left in a desiccator until it reached room temperature, then the constant weight was recorded [10].

2.9.2.2. Determination of Total Ash Content

1 g of extract is weighed and placed in a silicate crucible that has previously been incandescent and weighed. Then the extract is incandescent using a furnace slowly until

the charcoal runs out. Then cooled in a desiccator and weighed until the weight remains constant. Then the total ash content is calculated which is expressed in% (w/w) [10].

2.9.2.3. Ethanol Free Test

The ethanol free test is carried out using the following procedure. The extract is added with H_2SO_4 then added again with CH_3COOH, then heated. The test result is negative if there is no distinctive ester odor [9].

2.10. Avocado seed gel formulation

Table 1. Avocado seed gel formulation

Material name	F0	F1	F2	F3	Function
Seed Extract Avocado	-	5%	10%	15%	active ingredients
Carbopol 940	0,5	0,5	0,5	0,5	emulsifier
Metil paraben	0,2	0,2	0,2	0,2	preservative
Propilenglikol	15	15	15	15	moisturizer
TEA	2	2	2	2	pH neutralizer
Aquadest	Ad 100 ml	Ad 100 ml	Ad 100 ml	Ad 100 ml	solvent

In a blender, methyl paraben is dissolved in hot distilled water, then in a mortar, carbopol 940 is added, then stirred until it expands and forms a gel, then avocado seed extract is added according to the desired concentration and other ingredients such as propylene glycol as a humectant and TEA as previously dissolved in distilled water. heat and pH neutralizer Carbopol 940 [15].

2.11. Physical Quality Test

2.11.1. Organoleptic Test

The test is carried out by observing the shape, color and smell at room temperature.

2.11.2. Homogeneity Test

Test the homogeneity of the gel by smearing 0.5 g of the gel preparation on a glass object, then rubbing it on the glass surface. It is said to be homogeneous if there are no coarse grains on the glass surface [15].

2.11.2. pH Test

The pH test of the gel preparation is measured using a tool called a pH meter. The pH value requirement for the preparation that must be met is pH 4.5-6.5 [15].

2.11.3. Spreadability Test

A total of 0.5 g of the preparation was weighed then placed on a watch glass and covered with another watch glass and then given a weight of 50 g. The requirements for a qualifying spreadability test are 5-7 cm [15].

2.11.4. Adhesion Test

A total of 0.5 g of the gel preparation was placed on the object glass, then covered with another object glass, and given a weight of 1 kg for 3 minutes, then released the object glass, pulled using a rope that had been attached. The requirement for adhesion is more than one second [15].

2.11.5. Viscosity Test

A total of 50 ml of the gel preparation was put into a measuring cup and then the viscosity was measured using a viscometer brookfield (NDJ-8S®) with spindle no. 4 at a speed of 30 rpm. The viscosity requirement for a good gel preparation is 50-1000 dps [16].

2.12. Animal burn test

Before conducting the test, the rabbits were acclimatized for two weeks at the Pharmacology and Natural Product Isolation Laboratory at Universitas An Nuur. The rabbits used were active, exhibited normal behavior, had white fur, were of adult age, and weighed between 1.0 and 2 kg. Rabbits with anatomical abnormalities or those that died during the study were excluded. Three rabbits were used in this study. Burns were induced on the rabbits' backs by shaving the fur in five areas, followed by anesthesia using 0.2 cc of 2% lidocaine injected intradermally and waiting for 2-3 minutes. Then, a heated metal plate, preheated for 5 seconds, was applied to cause blistering. One rabbit's back was divided into 5 groups: Group I (negative control), Group II (positive control), Group III (5% ethanol extract of avocado seeds), Group IV (10% ethanol extract of avocado seeds), and Group V (15% ethanol extract of avocado seeds). Each wound was treated with gel twice daily for 10 days. Wound diameter was measured daily for 10 days, and the wounds were observed until healed, which was indicated by the disappearance of inflammation and scab shedding [17].

3. Results and Discussion

3.1. Results of Material Collection

Table 2. Dry weight results of avocado seed powder

Wet Weight (gr)	Dry Weight of Simple (gr)	Dry weight of powder (gr)	Presentation (%)
6000	4000	600	15

6000 gr of fresh avocado seeds are dried and produced into 4000 gr of simplex, then ground to obtain 600 gr of dry avocado seed powder with a percentage of 15%.

3.2. Results of Making Avocado Seed Extract

Table 3. Avocado seed extract yield results

Avocado seed powder (gr)	Thick extract (gr)	Yield (%)
600	101	16,83

Obtained a result of 16.83%. The yield value obtained is related to the number of secondary metabolite compounds in avocado seed extract. The higher the yield value, the more efficient the treatment applied. In addition, the greater the contact between the surface of the simplicia particles and the solvent, the easier it is for the solvent to draw compounds from the simplicia, so that the amount of extract obtained increases.

3.3. Results Simplicity Parameter Test

3.3.1. Specific

3.3.1.1. Organoleptic test

Table 4. Organoleptic test results

smell	shape	Flavor	color
distinctive smell	Fine powder	Bitter, astringent	Red slightly brown

3.3.2. Non Specific

Table 5. Total ash content and drying shringkage

Replication	Ash content results (%)	drying shringkage results (%)
Replication I	1,64 %	11,92%
Replication II	1,54 %	11,93%
Replication II	1, 63 %	11,76%
Average ash content ± SD	1,60 ± 0.07	11,87 ± 0,095

3.3.2.1. Total Ash Content

The results of this study showed that the ash content was 1.60±0.07%, this result is smaller than the Herbal Pharmacopoeia [18] requirements that the ash content in good powder is <2.3%. In a previous study, [19] reported an ash content of 2%, while in this study, the ash content was 1.60±0.07%. This result is lower than the previous study but still considered good as it is in accordance with the required standards.

3.3.2.2. Drying Shrinkage

According to the Herbal Pharmacopoeia [18], good drying shrinkage results are less than 19%. In a previous study, [20] reported that the drying shrinkage of avocado seed powder was 1.3%, while in this study, the drying shrinkage of avocado seed powder was significantly different, as it was 11.87%, which means it meets the standards according to the Herbal Pharmacopoeia (2017).

3.4. Result Parameter Extraction test

3.4.1. Specific

3.4.1.1. Organoleptic test

Table 6. Organoleptic test results

smell	shape	flavor	color
distinctive smell	thick	Bitter, astringent	dark chocolate

3.4.1.2. Test Results For The Compound Content Of Avocado Seeds

Table 7. Test results for the compound content of avocado seeds

No	Chemical Content	Reagent	Results	Conclusion
1	Flavonoid	Mayer	An orange-yellow precipitate occurs	(+)
2	Alkaloid	Wgner	An orange precitate occurs	(+)
3	Tanin	$Fecl_3$ 10%	A greenish block precipitate occurs	(+)
4	Saponin	HCl 2N	Foam forms	(+)
5	Triterpenoid	Liberman-burchad	Red precipitate occurs	(+)

Information :
(+) = Positive Result
(-) = Negative Result

The chemical content identification of avocado seed extract conducted in this study showed that the extract contains alkaloids, flavonoids, saponins, tannins, and triterpenoids. The results of this study are consistent with previous research by [21] who found that avocado seeds contain secondary metabolites such as alkaloids, flavonoids, saponins, tannins, and triterpenoids.

3.4.1.3. Results of Thin Layer Chromatography

Table 8. Results of thin layer chromatography

No	Chemical Content	eluent	Results
1	Alkaloid	Ethyl acetate: methanol: water (100:13.5:10)	(+)
2	Flavonoid	n-butanol: acetic acid: water (4:1:5)	(+)
3	Tanin	Methanol: water (6:4)	(+)
4	Saponin	Chloroform: acetone: (4:1)	(+)
5	Triterpenoid	n-hexane : ethyl acetate (5:5)	(+)

Information :
(+) = Positive Result
(-) = Negative Result

The chemical compound identification results showed that avocado seed extract contains alkaloids, flavonoids, saponins, tannins, and triterpenoids. The results of this study are in line with the research of [22], which reported the presence of chemical compounds in avocado seed extract. Avocado seeds (Syzgium polyanthum) contain flavonoids, saponins, and tannins [6].

3.4.2. Non Specific

Table 9. Extract ash content, result of drying shringkage, and ethanol free

Replication	Ash content results (%)	drying shringkage results (%)	Ethanol free
Replication I	1,00 %	14,91%	-
Replication II	0,90 %	16,30%	-
Replication II	1,09 %	14,57%	-
Average ash content ± SD	0,99 ± 0.55	15,26 ± 0,92	-

Information :
(-) = Negative Result

3.4.2.1. Extract Ash Content

The ash content test was carried out with the aim of providing an overview of the internal and external mineral content originating from the initial process resulting in the formation of an extract according to the Indonesian Ministry of Health [23], In previous research, Khofifah [20] stated that the ash content was 3.5% and in the results of this study the ash content was 0.99%, this result is smaller than previous research but is still good because it still complies with the provisions.

3.4.2.2. Drying Shrinkage

The ash content of the extract complies with the FHI (2017) standards, which state that the ash content in a good extract should be < 2.3%. The ash content test is conducted to provide an overview of the internal and external mineral content that originates from the initial processing, which contributes to the formation of the extract, according to the Ministry of Health of the Republic of Indonesia (Depkes RI) in [10] In a previous study, Khofifah et al. (2023) reported an ash content of 3.5%, while in this study, the ash content was 0.99%. This result is lower than the previous study but still considered good as it complies with the standards [20].

3.4.2.3. Ethanol Free

The ethanol-free test results in this study showed that the avocado seed extract was free from its solvent, 96% ethanol, as indicated by the absence of the characteristic ester smell of ethanol. The results of a previous study by [20] stated that the ethanol-free test showed no ester smell, meaning that the ethanol extract of avocado seeds was positively free from the ethanol solvent.

3.5. Gel Preparation Results

The avocado seed extract gel preparation formula is made into 4 different formulas. The difference in formula is in the amount of extract added. Formula 0 without using extract (negative control), Formula 1 with the addition of 5% extract, Formula 2 10% extract and Formula 3 15% extract.

3.6. Physical Quality Evaluation Results

3.6.1. Organoleptic Test

Table 10. Organoleptic test results of avocado seed extract gel preparation

Formula	Test Result		
	smell	shape	color
F0	no smell	Semi solid	colorless
F1	distinctive smell of avocado	Semi solid	Reddish brown
F2	distinctive smell of avocado	Semi solid	Reddish brown
F3	distinctive smell of avocado	Semi solid	brown

The results obtained were that from the three preparations there were no significant changes. All formulations have a texture consistency, namely semi-solid. It is brownish red in color. the more extract you add, the more brown the resulting preparation will be.

3.6.2. pH Test

Table 11. Results of pH, homogenity, adhesion, spreadability, viscosity test avocado seed extract gel preparation

No	Formula	pH Test Average ± SD	Homogenity test	Adhesion Test Average ± SD (second)	Spreadability Test Average ± SD	Viscosity Test Average ± SD
1	F0	4.35 ± 0.02	Homogeneous	04.64 ± 0.57	4.83 ± 1.27	252.67 ± 2.52
2	F1	5.29 ± 0.02	Homogeneous	15.85 ± 1.39	5.47 ± 1.15	113.33 ± 11.55
3	F2	6.07 ± 0.02	Homogeneous	10.83 ± 0.26	6.23 ± 0.75	91.67 ± 2.89
4	F3	6.35 ± 0.02	Homogeneous	08.34 ± 0.42	6.73 ± 0.50	62.33 ± 2.08

Previous research by Satolom [24] stated that the pH of avocado seed extract obtained in testing avocado seed gel was 5.93. Based on the preparations that have been tested, it can be concluded that avocado seed extract gel with varying concentrations meets the appropriate pH requirements and is permitted on human skin. According to Aprilianti [25] human facial skin has a pH value of between 4.5-6.5.

3.6.3. Homogenity test

A preparation is said to be homogeneous when there are no visible coarse grains or particle spots. The results of the homogeneity test, where in gel preparations with varying concentrations, a homogeneous preparation was obtained and no particle spots were visible.

3.6.4. Adhesion Test

The test results in this research showed that the adhesion of the avocado seed ethanol extract gel had an average of 4.64 - 15.85 seconds, meaning that in this study the results were greater than previous research and met the requirements.

3.6.5. Spreadability Test

The results of testing the spreadability of avocado seed ethanol extract gel in this study had a spreadability of 4.83-6.73 cm. This result was also influenced by the small viscosity value. Thus, the spreadability of the Avocado seed extract gel preparation is safe for use.

3.6.6. Viscosity Test

A good gel viscosity value is in the range of 50-1000 dPa.s [26]. Based on the results of the viscosity tests carried out, it shows that the avocado seed extract gel preparation has good viscosity values, namely F0 of 252.67 dPa.s, F1 of 113.33 dPa.s, F2 of 91.67 dPa.s, and F3 of 62.33 dPa.s. The results of this research show that the higher the concentration of avocado seed extract, the lower or lower the viscosity.

3.7. Activity Test Results of Avocado Seed Extract Gel Preparation *(Persea americana Mill.)* on Healing Burns on the Backs of New Zealand Rabbits

This test is aimed at finding out at what concentration avocado seed extract gel can provide the fastest healing effect on burn wounds.Gel is applied twice a day (morning and evening), before the wound must be cleaned before applying the gel. Each rabbit received 5 treatments.

Table 12. Percentage of healing of burn wounds on the backs of new zealand rabbits

Day	Wound Healing Presentation (%)				
	K+	F0	F1	F2	F3
1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	18.50±15.99	3.30±5.63	6.50±5.63	12.80±5.34	12.80±0.05
3	54.10±18.07	21.60±13.31	15.70±10.36	27.60±8.50	30.10±0.13
4	60.80±23.58	35.50±13.45	21.60±13.31	27.50±23.63	47.80±0.15
5	81.20±10.80	40.50±15.49	35.50±13.45	59.50±9.47	65.60±0.09
6	87.50±10.80	49.80±17.66	55.30±7.51	72.60±10.30	77.50±0.09
7	96.60±3.19	59.00±13.86	72.60±10.30	84.20±9.35	89.20±0.06
8	97.30±2.31	77.70±13.86	88.60±7.91	92.60±5.81	97.00±0.03
9	99.30±0.58	89.00±8.66	94.60±4.07	97.90±2.01	99.60±0.01
10	99.70±0.58	94.90±4.62	97.90±2.01	99.30±0.58	99.90±0.00

Information :
F0 = Base
K+ = Gel containing 10% placenta extract and 0.5% neomycin sulfate positive control
F1 = ethanol extract 96% avocado seeds 5%
F2 = 96% ethanol extract of 10% avocado seeds
F3 = ethanol extract 96% avocado seeds 15%

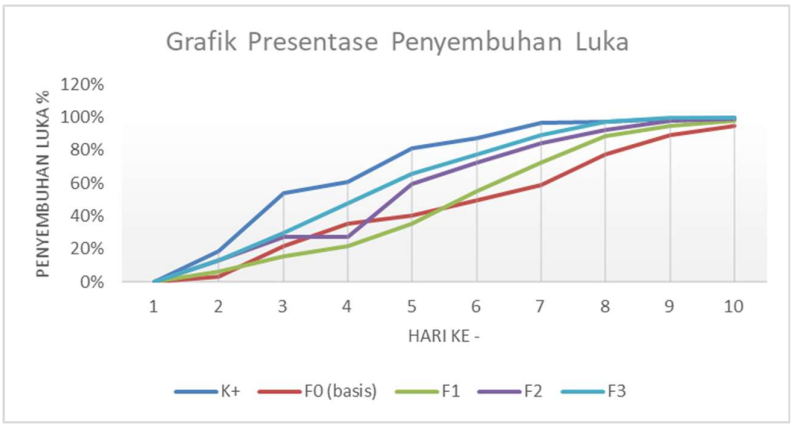

Figure 1. Graph of the percentage of healing of burn wounds on the backs of New Zealand Rabbits

From data from wound measurements on the healing process of burns in New Zealand rabbits weighing 1-2 kg for 10 days. Wound diameter measurements for all treatment groups on days 1 to 10 experienced changes. On day 1, for all treatment groups, slight changes were visible on the edges of the burn wounds. On day 5, most wounds from all treatments began to dry out, while on day 10, all wounds had healed significantly. In the treatment with avocado seed extract gel *(Persea americana Mill.)* with a concentration of 15%, it healed and dried completely, compared to the other treatment groups. Thus, the avocado seed extract gel preparation *(Persea americana Mill.)* contains active substances that can be formulated into an avocado seed extract gel preparation for healing burns in New Zealand rabbits. This is because it can have a healing effect on burn wounds in rabbits, an effect that is almost the same as the positive control. Meanwhile, avocado seed extract gel *(Persea americana Mill.)* provides the best effect at a concentration of 15%.

The wound healing process using avocado seed extract occurs due to the secondary metabolites in the avocado seeds, namely flavonoids, which have the ability to accelerate wound healing and exhibit antibacterial activity by forming complex compounds on extracellular proteins that can disrupt the integrity of bacterial cell membranes. Flavonoids work by preventing blood vessel blockages, improving circulation throughout the body, and having anti-inflammatory, antioxidant properties while reducing pain in inflamed and bleeding areas [27]. Additionally, flavonoids function as anti-inflammatory agents by scavenging free radicals that trigger inflammatory responses, and these compounds can be categorized as antioxidants [28]. According to a 2016 study, the saponin compounds play a role in fibroblast proliferation because saponin has antibacterial activity. Saponin also activates the functions of transforming growth factor β (TGF-β), vascular endothelial growth factor (VEGF), and fibroblast growth factor-1 (FGF-1). TGF-β and FGF-1 stimulate fibroblast migration and proliferation [29]. Furthermore, a 2018 in vitro study revealed that saponin can inhibit bacterial growth by lowering the surface tension of bacterial cell walls and damaging membrane permeability due to its soap-like properties [30]. Another compound believed to play a role in fibroblast proliferation is tannin. Tannin acts as an antioxidant and can induce TGF-β to support fibroblast proliferation[29]. Tannin also induces lymphokines to enhance macrophage migration, which then stimulates the secretion of growth factors, boosting fibroblast proliferation. Fibroblasts are crucial for tissue repair, from the inflammatory phase to the final epithelialization of the injured tissue, by releasing growth factors, cytokines, collagen, and other extracellular matrix (ECM) components. The migration and proliferation of fibroblasts are key in initiating the proliferative phase of wound repair [31]. Triterpenoids are known to promote fibroblasts that will produce collagen, supporting the structure of the area undergoing wound healing [32]. Alkaloids, which contain at least one nitrogen atom in a heterocyclic ring structure, function as antimicrobial agents and antioxidants [33]. Alkaloids are also essential in the healing of burn wounds due to their antibacterial properties [1]. In addition to secondary metabolites, the process of burn wound healing is influenced by various other factors, including proper care, such as providing sufficient food, maintaining cleanliness of the rabbit and its cage, and regularly applying avocado seed gel every morning and evening.

4. Conclusion

Based on the results of the study on the effect of 96% ethanol extract gel of avocado seeds (Persea americana Mill.) on burn wound healing on the backs of New Zealand rabbits, it can be concluded that the gel formulation used has an impact on the effectiveness of burn

wound healing, with a 15% concentration being the most effective. This 15% concentration is the highest because the 96% ethanol extract of avocado seeds (Persea americana Mill.) contains alkaloids, flavonoids, saponins, tannins, and triterpenoids.

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