TRENDS IN SCIENCES 2025; 22(11): 10453
https://doi.org/10.48048/tis.2025.10453 RESEARCH ARTICLE

Antibacterial and Wound Healing Activities of Oral Suspension and Cream of Ethanol Extract of Lemon Peel (*Citrus limon* (L.)) Burm.f.

Risfi Dara Saputri[1], Yuandani[1,*], Aminah Dalimunthe[1], Abdi Wira Septama[2] and Lisda Rimayani Nasution[3]

[1]*Department of Pharmacology and Clinical/Community Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia*
[2]*Research Center for Pharmaceutical Ingredient and Traditional Medicine, National Research and Innovation Agency (BRIN), KST Soekarno, Cibinong, Jawa Barat 16911, Indonesia*
[3]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia*

([*]Corresponding author's e-mail: yuandani@usu.ac.id)

Received: 24 April 2025, Revised: 25 May 2025, Accepted: 10 June 2025, Published: 5 August 2025

Abstract

The purpose of this study was to determine the antibacterial activity of ethanol extract of lemon peel against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa bacteria* as well as Healing of excision wounds from suspension and cream preparations of lemon peel ethanol extract. The antibacterial activity of lemon peel ethanol extract was evaluated against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* bacteria using the disc diffusion method. The lemon peel ethanol extract with the best antibacterial activity was formulated into a cream dosage form with variations in concentrations of F1 (10%), F2 (20%), and F3 (40%). Then, the excision wound healing activity was tested using the oral, topical, and combined routes (suspension and cream of lemon peel ethanol extract). The treatment of excision wound healing activity was carried out for 16 days with observations of the reduction in wound diameter on days 0, 3, 7, 12, and 16. Ethanol extract of lemon peel has antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* bacteria with an inhibition zone diameter of 9.4 mm for *Escherichia coli* bacteria. *Staphylococcus aureus* (11.0), and *Pseudomonas aeruginosa* (11.2 mm) with a concentration of 40%. Lemon peel ethanol extract cream was stable during 12 weeks of storage. Treatment with a combined oral and topical route (suspension and cream of lemon peel ethanol extract) had better wound healing activity for excision compared to oral and topical routes. The results showed that the suspension and cream of lemon peel ethanol extract have the potential to heal excision wounds.

Keywords: Lemon peel, Cream, Excision wound, Wound healing

Introduction

Wounds are a form of tissue damage to the skin caused by physical contact (with a heat source), the result of medical treatment, or changes in physiological conditions. When a wound occurs, the body naturally performs a wound healing process through biocellular and biochemical activities that occur continuously. The wound healing process consists of a series of inflammatory, proliferative, and remodeling phases [1]. The most frequently isolated pathogenic bacteria in cases of sepsis and wound infections are *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Lemon peel consists of 2 layers, the outer layer and the inner layer**.** The outer layer contains essential oils consisting of citral (5%) and limonene, α-terpineol, geranil acetate, and linalool. The inner layer contains coumarins, glycosides, and flavonoids [11]. The

bioactive compounds contained in lemon each have antibacterial properties [15]. Other active compounds such as eriocitrin, hesperidin, neohesperidin, diosmin, rutin, luteolin, nobiletin, sinensetin, and tangeritin [27] are flavonoid derivatives. Flavonoids can inhibit platelet attachment, aggregation, and secretion [26]. Flavonoids work in all phases of wound healing, such as the inflammatory phase, proliferation phase, and remodeling phase [10]. Flavonoids can reduce pain in the wound, reduce lipid peroxidase by increasing vascularization, thereby preventing necrosis [8].

Based on the description above, with the increasingly busy society and the rapid advancement of technology, people tend to use everything practical. This underlies researchers to innovate to create products that can fulfil the wishes of the community. One product that can be developed by researchers is a cream preparation in wound treatment based on natural ingredients to facilitate the community in its use. This cream has several advantages, namely easy multiplication, comfortable when used on the skin, non-sticky, and easy to wash water, especially oil-in water (O/W) creams [22].

Materials and methods

Materials

The materials used in this study were *Citrus limon* (L.) Burm.f. peel, ethanol 96% (pro analysis grade), *Staphylococcus aureus (*ATCC® 25923™), *Escherichia coli* (ATCC® 25922™), and *Pseudomonas aeruginosa (*ATCC® 27853™).

Plant collection and identification

Citrus limon (L.) Burm.f. were collected from Lau Chi Advertise Medan, North Sumatra. Plant was determined by Medanense (MEDA) Herbarium, Faculty of Mathematics and Natural Science, University of North Sumatra (Voucher No. 1816/MEDA/2024). The fresh lemon peel was sorted, thoroughly cleaned, cut into smaller pieces, and dried in an oven maintained at 40 - 45 °C for several days. The dried lemon peel was then ground to produce a fine powder.

Extraction procedure

The fine powder of crude plant of *Citrus limon* (L.) Burm.f. was extracted by maceration using ethanol 96% (pro analysis grade). Extract was concentrated by rotary evaporator, and the viscous extract was stored in a closed container at room temperature and protected from the light.

Phytochemical screening

Qualitative phytochemical identification was conducted to determine the chemical constituents of *Citrus limon* L. peel extracts, including flavonoids, alkaloids, saponins, tannins, glycosides, and steroids/ terpenoids.

Antibacterial activity of lemon peel

Antibacterial activity testing was performed using the paper disc diffusion method with concentrations of 100, 200, and 400 mg/ mL by using 3 pathogenic bacteria: *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* gotten from the Microbiology Laboratory of the Faculty of Pharmacy, University of North Sumatra.

Table 1 Formula of cream preparation of ethanol extract of lemon peel.

	Formulas (%)				
Materials	**F0**	**FI**	**FII**	**FIII**	**Function**
Lemon peel ethanol extract	-	10	20	40	Active substance
Adeps lanae	3	3	3	3	Emollient
Liquid parafin	15	15	15	15	Humectant
TEA (trietanolamin)	3	3	3	3	Emulgator
Stearic acid	14.5	14.5	14.5	14.5	Emulgator
Propyl paraben	0.05	0.05	0.05	0.05	Preservative

	Formulas (%)				
Materials	**F0**	**FI**	**FII**	**FIII**	**Function**
Methyl paraben	0.1	0.1	0.1	0.1	Preservative
Aquadest	100	100	100	100	Solvent

Lemon peel extract cream formulation

The cream formulation was prepared according to a previous research conducted by Wahyuni *et al.* [39]. The formula of the ingredients used in this study for the cream can be seen in **Table 1**.

Cream preparations of ethanol extract of lemon peel were prepared by heating the oil phase (stearic acid, liquid paraffin, adeps lanae, and propyl paraben) and water phase (TEA and methyl paraben) respectively on a water bath at 70°C. Next, the oil phase was transferred into a mortar containing the water phase, then distilled water, and stirred until homogeneous until a creamy mass. Then, the cream base is mixed with ethanol extract of lemon peel with the best concentration, then stirred until homogeneous [24].

Cream evaluation test

Cream evaluation test includes organoleptic test, homogeneity, pH, cream type, viscosity, spreadability, and adhesion test.

Cycling test

The cream was stored at 4 ℃ for 24 h (1 cycle), and 40 ℃ for 24 h (1 cycle), repeated for 6 cycles, and then organoleptic observations were made.

Animals

Wistar male rats weighing 150 - 200 g were used. Animal Research Ethics Committees (AREC) , University of North Sumatra (0764/ KEPH-FMIPA/2024 , evaluated all procedures.

Excision wounds in animals

Wounds were made in the back area of the rats after being anesthetized with ketamine 50 mg/ kg intraperitoneally [8]. Then labeled with a permanent marker, a circular excision wound with a diameter of 2 cm is made using a sterilized biopsy punch. The day when the wound was made was considered wound day 0.

Wound healing activity

The wound healing test was given topically with ethanol extract cream of lemon peel; namely, rats were divided into 5 groups of 5 each. The first group was a negative control applied cream base, group 2 was a positive control with silver sulfadiazine cream, and groups 3, 4, and 5 were applied lemon peel extract cream 10%, 20%, and 40%.

The oral groups were administered with lemon peel ethanol extract suspension with CMC Na 0.5% as a suspending agent. The first group was a negative control that was given a 0.5% CMC Na suspension, group 2 was a positive control with a cork fish extract suspension, groups 3, 4, and 5 were given a suspension of lemon peel extract doses of 100, 200, and 400 mg/kgBW.

Combination of suspension and cream of ethanol extract of lemon peel. The first group was a negative control given a combination of cream base and 0.5% CMC Na suspension, group 2 was a positive control with sulfadiazine cream and Hyaluronic acid suspension, and groups 3, 4, and 5 were given lemon peel extract cream concentrations of 10%, 20% and 40% and suspense of lemon peel extract doses of 100, 200, and 400 mg/kgBW, respectively [2].

Wound area measurement

Observations and measurements of wound reduction diameter were made on days 0, 3, 7, 12, and 16 by measuring the diameter of the wound using the *ImageJ* application [18].

$$\%\text{wound healing} = \frac{\text{wound 0} - \text{wound n}}{\text{wound 0}} \times 100\%$$

Histopathology study

On postoperative day 16, excised skin tissues from all treatment route groups were subjected to histopathological examination. All samples were fixed in 10% buffered formalin, blocked with paraffin, and then sectioned at 5 μm thickness. All sections were

stained with hematoxylin and eosin (H-E) dye [29]. The stained tissues were examined and photographed using a Nikon digital microscope.

Statistical analysis

The results were subjected to an ANOVA test by determining significant differences and expressed as mean ± SD ($p < 0.05$). The significance between groups was analyzed using the Duncan test.

Results and discussion

Preparation of lemon peel extract

Lemon (*Citrus limon* (L.)) Burm.f. was obtained from Lau Chi Market Medan, North Sumatra. The extract obtained from the maceration process of lemon peel is 133.4 g, so the extract yield is (15.24%).

Phytochemical screening

Based on the results of phytochemical screening, it was known that the extract of lemon peel contains alkaloids, tannins, saponins, triterpenoids/ steroids, glycosides, and flavonoids.

Antibacterial activity of extract

Based on the results of testing the antibacterial activity of ethanol extract of lemon peel shows that the largest diameter to inhibit the growth of *Escherichia Coli* bacteria is at 40% extract concentration, with an inhibition zone diameter of 9.4 mm, with a fairly active category. While in *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria, there is a 40% extract concentration with an inhibition zone diameter of 11.0 mm (*Staphylococcus aureus*) and 11.2 mm (*Pseudomonas aeruginosa*), with a strong category. The smallest concentration of the 3 bacteria was shown at a concentration of 10% with an inhibition zone diameter of <10 mm, which included the fairly active category. The results of the antibacterial activity test on lemon peel extract aim to determine the concentration of extracts used in making cream preparation.

Characteristic and stability evaluation of *Citrus limon* cream extract

Organoleptic evaluation

The resulting lemon extract cream formulation was semi-solid, light yellow to brown in color, and had a distinctive lemon odor. The difference in color is due to the different concentrations of extracts used; the higher the concentration, the more extracts are added, resulting in a darker cream color gradient.

Homogeneity evaluation

Homogeneity in all cream preparations shows homogeneous results, characterized by all particles that are evenly dispersed on the glass object and there is no clumping in each preparation.

pH evaluation

Based on the pH evaluation results that the 3 formulas have different pH where F1 has a pH of 6.07, F2 has a pH of 5.86, and F3 has a pH of 5.62. The difference in pH can occur because the smaller the amount of stearic acid and the greater the addition of TEA can cause an increase in the pH value of the cream. Due to the alkaline nature of TEA, the addition of TEA, which can neutralize stearic acid, causes a cream pH that was close to alkaline [12].

Cream type evaluation

Extract cream is a type of water-in-oil (w/o) cream characterized by uneven or separate methylene blue color results with the cream base. This can occur because the methylene blue base is a water base while the cream base is an oil base.

Viscosity evaluation

Based on storage data in the 12th week, the viscosity value of 40% lemon peel extract cream was 3,054 cP. it shows that the viscosity value of the cream preparation still meets the SNI requirements, namely in the range of 2,000 - 50,000 cP.

Evaluation of spreadibility

Based on the results of the spreadability test in that the diameter of the spread of cream preparations containing ethanol extract of lemon peel is greater than the cream base made. This shows that the addition of the extract affects the consistency of the cream to be more liquid so that the spreadability of the preparation becomes greater, but from the results obtained, both the base and the cream with the extract have good spreadability. The appropriate spreadability diameter for semi-solid preparations is 5 - 7 cm [14].

Evaluation of adhesion

The adhesion time of F1 is greater than the adhesion time of F2, and F3. This is because the adhesion of the cream preparation is directly proportional to the viscosity or viscosity of the preparation. So that the higher the viscosity value, the longer the resulting adhesion time. Adhesion time affects the length of contact of the cream with the skin. If the adhesion is too strong it can interfere the skin function, while if it is too weak it will reduce the therapeutic effect [9].

Stability evaluation

The stability evaluation was conducted using cycling test. The stability of the formulation was determined by the characteristic of the formulation, including organoleptic, pH, and viscosity properties as shown us **Tables 2 - 4**.

The results of pH stability measurements in **Table 2** show that during the 12-week storage period, cream preparation formulations F0, F1, F2, and F3 experienced a slight decrease in pH value. However, it is still within the range that corresponds to the skin's pH, so it can be used and has no adverse effect on the skin.

The results of viscosity stability measurements in **Table 3** show that during the 12-week storage period, cream preparation formulations F0, F1, F2, and F3 experienced a slight decrease in viscosity value. However, it is still within the range that corresponds to the cream preparation's viscosity, so it can be used and has no adverse effect on the skin.

Table 2 pH of *Citrus limon* cream extract.

Weeks	Measurement Results (pH)			
	F0	F1	F2	F3
0	6.33	6.07	5.86	5.62
12	5.39	5.29	5.19	5.09

Table 3 Viscosity of *Citrus limon* cream extract.

Weeks	Measurement Results (cPs)			
	F0	F1	F2	F3
0	4.709	3.780	3.396	3.054
12	4.678	3.753	3.365	3.029

Table 4 Organoleptic examination of *Citrus limon* cream extract.

Observation		Formulas			
		F0	F1	F2	F3
Color	Before	White	Light yellow	Light brown	Brown
	After	White	Light yellow	Light brown	Brown
Odor	Before	Odorless	Characteristic	Characteristic	Characteristic
	After	Odorless	Characteristic	Characteristic	Characteristic
Form	Before	Semi solid	Semi solid	Semi solid	Semi solid
	After	Semi solid	Semi solid	Semi solid	Semi solid
Separation phase	Before	-	-	-	-
	After	-	-	-	-

The results of cycling test in **Table 4** show that all the cream formulations before testing have no clouds/crystals. After completion of the cycling test, the cream was observed again. The results of observations on all cream formulations did not form phase separation and did not form crystallization. In this cycling test,

there is no phase separation or crystallization, which means that the emulsifying film contained in the cream can prevent coalescence caused by cold temperatures.

Antibacterial activity test results of lemon peel extract cream preparations

Based on the results of the diameter of the inhibition zone of the cream preparation of ethanol extract of lemon peel that the largest diameter to inhibit the growth of *Escherichia coli* bacteria is in the concentration of 40% cream preparation with an inhibition zone diameter of 7. 03 mm with a fairly category. While in *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria, there is a 40% concentration of cream preparation with an inhibition zone diameter of 9. 5 mm (*Staphylococcus aureus*) and 12. 2 mm (*Pseudomonas aeruginosa*) with a strong category. The smallest concentration of the 3 bacteria was shown at a cream preparation concentration of 10% with an inhibition zone diameter of <9 mm which is included in the fairly active category.

Wound healing activity

Observation of the effectiveness of wound healing is done visually, where the wound healing process is seen from changes in the diameter of the wound which is getting smaller and the percentage of wound diameter reduction is increasing. The percentage of wound healing for lemon peel extract suspension administered orally, lemon peel extract cream administered topically, and suspension and cream administered in combination can be seen in **Table 5.**

Table 5 Percentage of wound healing.

Groups	Percentage of Cure ± SD Days of Observation				
	0	**3**	**7**	**12**	**16**
Oral					
Lemon peel extract 100 mg/kgBW	0 ± 0.00	8.5 ± 0.79^{a,b}	18 ± 0.81^{a,b}	52.5 ± 1.10^{a,b}	63 ± 1.46^{a,b}
Lemon peel extract 200 mg/kgBW	0 ± 0.00	12.5 ± 1.90^{a,b}	22.5 ± 1.67^{a,b}	57.5 ± 1.84^{a,b}	6.5 ± 2.85^{a,b}
Lemon peel extract 400 mg/kgBW	0 ± 0.00	18 ± 2.21^{a,b}	28 ± 2.20^{a,b}	63 ± 2.19^{a,b}	72.5 ± 2.18^{a,b}
Cork Fish Extract Suspension	0 ± 0.00	22 ± 0.95^{b}	32.5 ± 0.99^{b}	67 ± 0.72^{b}	77 ± 1.33^{b}
CMC Na 0.5%	0 ± 0.00	6.5 ± 0.58^{a}	12 ± 1.50^{a}	46.5 ± 1.40^{a}	57.5 ± 1.29^{a}
Topical					
Lemon peel extract cream 10%	0 ± 0.00	13 ± 1.88^{a}	22.5 ± 3.17^{a,b}	58 ± 0.18^{a,b}	69 ± 1.87^{a,b}
Lemon peel extract cream 20%	0 ± 0.00	20 ± 1.17^{a,b}	28.5 ± 1.43^{a,b}	63 ± 2.19^{a,b}	74 ± 1.22^{a,b}
Lemon peel extract cream 40%	0 ± 0.00	23.5 ± 0.73^{a,b}	32.5 ± 3.14^{a,b}	68.5 ± 1.57^{a,b}	77 ± 0.67^{a,b}
Burnazin Cream	0 ± 0.00	27.5 ± 3.13^{b}	37.5 ± 1.44^{b}	74 ± 2.15^{b}	89 ± 0.11^{b}
Cream Base	0 ± 0.00	12 ± 1.19^{a}	17.5 ± 1.11^{a}	44 ± 1.98^{a}	62.5 ± 1.35^{a}
Combination					
Cream 10% and 100 mg/kgBW	0 ± 0.00	19.5 ± 3.12^{a,b}	29 ± 0.99^{a,b}	64.5 ± 2.12^{a,b}	74 ± 1.12^{a,b}
Cream 20% and 200 mg/KgBW	0 ± 0.00	24 ± 1.11^{a,b}	33 ± 3.17^{a,b}	67 ± 1.23^{a,b}	78 ± 0.13^{a,b}
Cream 40% and 400 mg/KgBW	0 ± 0.00	27.5 ± 1.18^{a,b}	38.5 ± 0.92^{a,b}	72.5 ± 1.61^{a,b}	83 ± 3.18^{a,b}
Hyaluronic acid Suspension and Burnazin Cream	0 ± 0.00	33 ± 3.16^{b}	43 ± 1.92^{b}	77 ± 0.47^{b}	93 ± 1.15^{b}
CMC Na 0.5% and Cream Base	0 ± 0.00	17.5 ± 0.63^{a}	21.5 ± 1.13^{a}	48 ± 1.09^{a}	68 ± 0.13^{a}

Description: a = there is a significant difference with the positive control group ($p < 0.05$), b = there is a significant difference with the negative control group ($p < 0.05$).

Based on **Table 5** shows that the results of the percentage of wound healing from the oral route (ethanol extract of lemon peel) , topical (cream of ethanol extract of lemon peel), and combined route it can be concluded that the administration of the combined route has a higher percentage of healing compared to the administration of the oral and topical route so it can be said that the combined route is more effective in healing excision wounds in white rats**.**

Wound healing always begins with the process of blood clotting, which has the aim of closing the wound [16] . Reddish discoloration of the wound indicates wound healing in the inflammatory phase. The inflammatory phase occurs immediately after the occurrence of the wound until the fifth day. In the inflammatory phase, cell membrane permeability occurs, resulting in inflammation, redness, heat, and pain [6]. In this phase, hemostatic events occur, assisted by fibrin threads so that red blood cells and plasma will be netted to form clots. These clots will form scabs [35].

Scab formation indicates that the healing phase process is entering the proliferation phase [6] . The proliferation or fibroplasia phase lasts for 3 weeks. The proliferation phase is assisted by fibroblasts, which are cells that produce collagen [35]. Collagen will work to connect tissue in the wound to restore skin tissue and accelerate wound healing [6].

The last stage is the maturation (remodeling) phase. The remodeling or maturation phase lasts from several weeks to 2 years and seeks to restore normal tissue structure. The maturation phase begins when the scab is removed and new skin tissue is seen [35]. Scab detachment occurs because the underlying tissue is dry and the edges of the wound begin to pull towards the center [5,35]. There are several chemical compounds that play a role in the wound healing process, such as flavonoids. Flavonoids are compounds that function as antioxidants by neutralizing Reactive Oxygen Species (ROS). When a wound occurs, there is an inflammatory process characterized by the activation of neutrophils and macrophages [23]. Activation of neutrophils and macrophages produces free radicals called Reactive Oxygen Species.

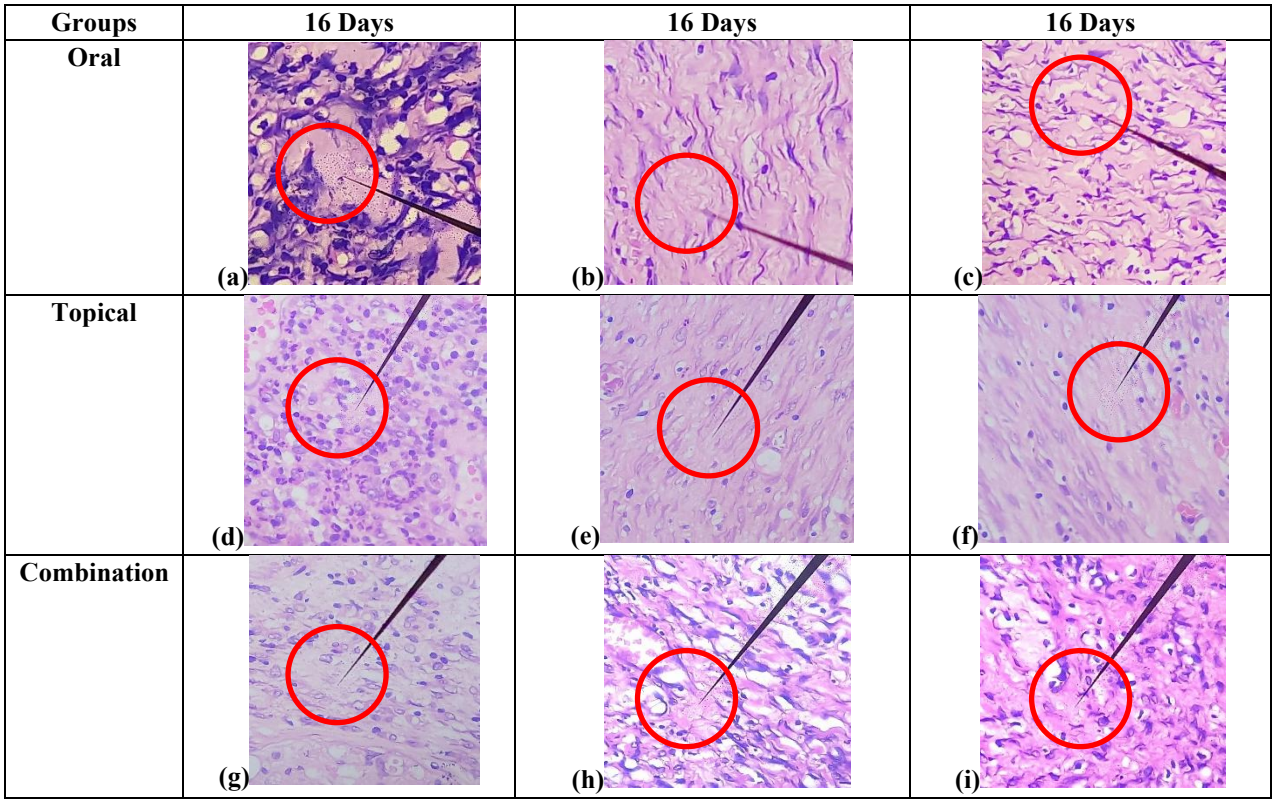

Figure 1 Collagen fiber of (a) CMC Na 0.5% suspension (b) Cork fish extract (c) Lemon peel extract 400 mg/kgBW (d) Base cream (e) Burnazine cream (f) Lemon peel extract cream 40% (g) Combination of CMC Na 0.5% and Base cream (h) Combination of Burnazine cream and *Hyaluronic acid* (i) Lemon peel extract 400 mg/kgBW suspension and cream 40%.

Histopathology examination

After wound closure and observation for 16 days, a histopathology study was done to observe the wound tissue after wound closure. Tissues cut from the positive control group from both oral, topical, and combined routes illustrated significant healing properties followed by fibroblast cells, collagen fibers, and well- formed angiogenesis. Tissues cut in the negative control of all groups of the route of administration on day 16 showed fibroblast cells, collagen fibers, and angiogenesis were not well formed. Then animals treated with lemon peel extract topically, orally, and in combination on day 16 also showed significant healing activity ($p < 0.05$) which could be caused by regeneration of fibroblast cells, angiogenesis, and collagen fibers.

Based on **Figure 1** shows the results of hispatology of collagen fiber formation after oral route administration in topical route and combined route. Collagen is the main fibrous protein of the extracellular matrix which is composed of 3 separate peptide chains and woven into a triple twist resembling a rope where the peptide chain has glycine in every third position which is able to provide a tight braid on each chain. Collagen plays an important role in the wound healing process. Collagen functions for hemostatis, interacts with platelets and fibrinectin, increases fluid exudation, increases cellular components, increases growth factors, encourages the fibroplasia process and plays a role in epidermal proliferation [30].

Statistical results with normality test showed normally distributed data ($p > 0.05$), where Snakehead Fish Extract was significantly different from CMC Na 0.5%, but not significantly different from the extract group with a dose of 400 mg/kgBW and extract dose of 200 mg/ kgBW. While the group given Lemon peel extract cream, Burnazin cream did not differ significantly from the Lemon peel extract Cream 40% group **(Figure 2)**.

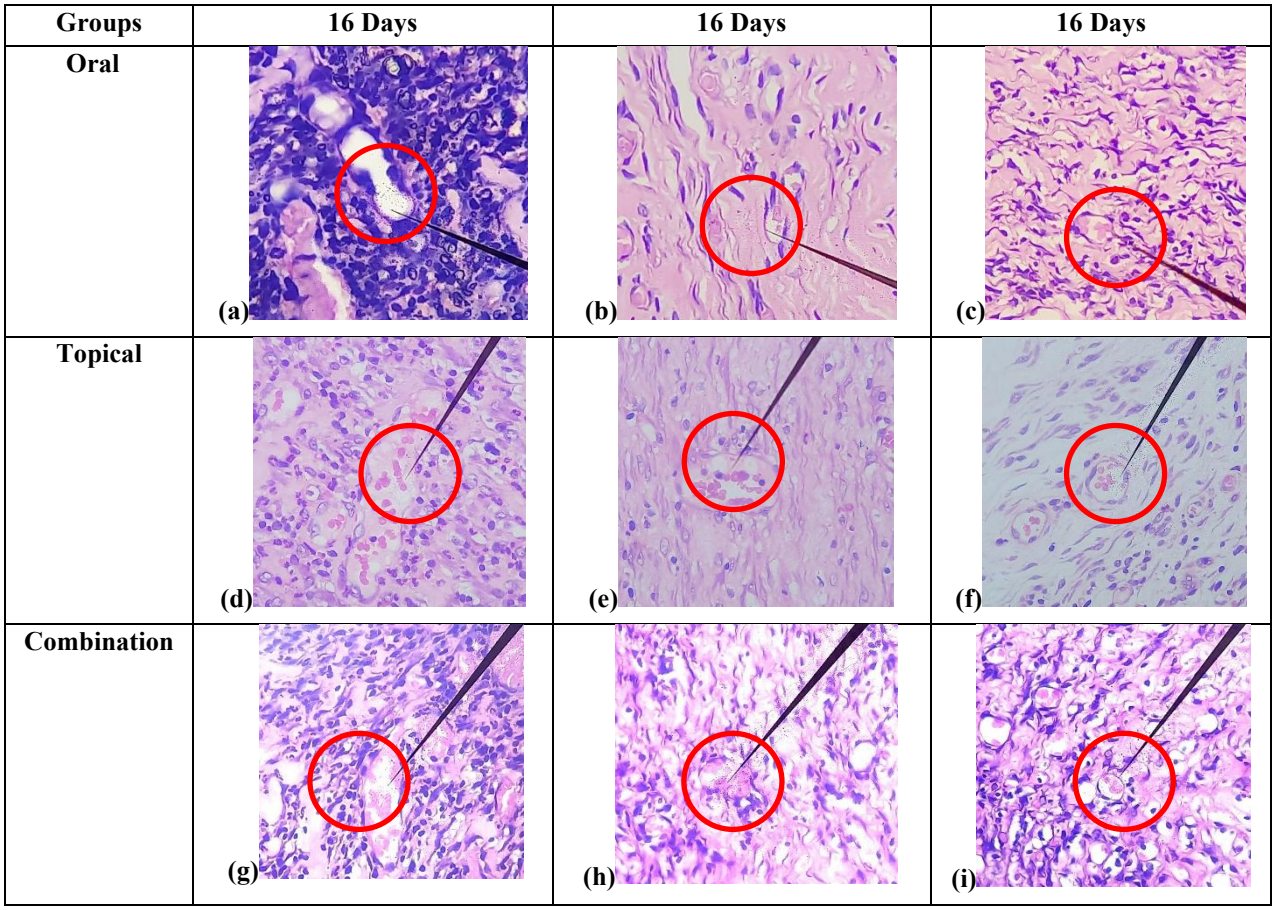

Figure 2 Angiogenesis of (a) CMC Na 0.5% suspension (b) Cork fish extract (c) Lemon peel extract 400 mg/kgBW (d) Base cream (e) Burnazine cream (f) Lemon peel extract cream 40% (g) Combination of CMC Na 0.5% and Base cream (h) Combination of Burnazine cream and *Hyaluronic acid* (i) Lemon peel extract 400 mg/kgBW suspension and cream 40%.

Angiogenesis is one aspect of repair that has always been considered essential for adequate healing is the creation of new blood vessels through angiogenesis. Angiogenesis is the process of forming new blood vessels from pre-existing blood vessels. The presence of blood vessels in the wound serves as transportation for the supply of nutrients and oxygen needed by the cells that are in the process of repair, to destroy waste substances and form granulation tissue [38].

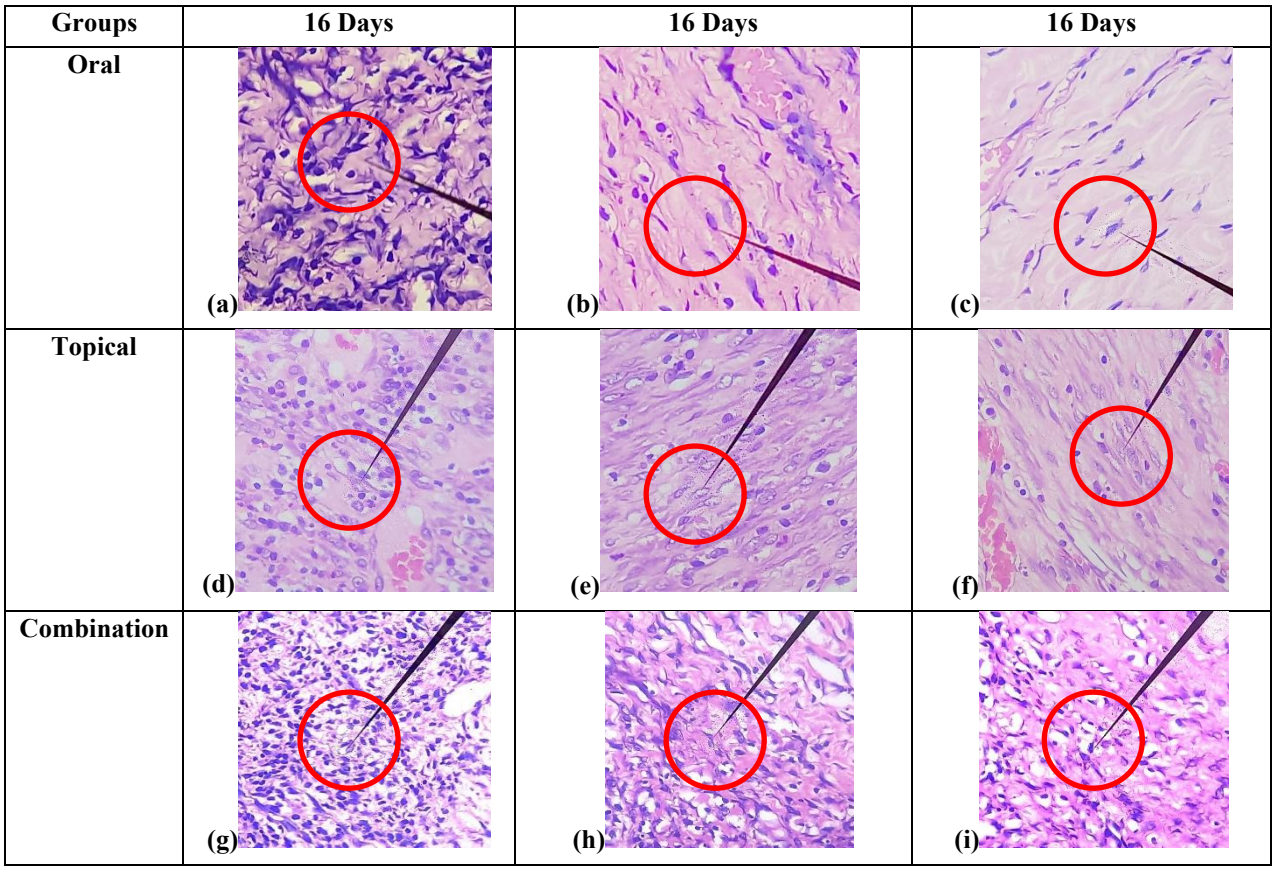

Figure 3 Fibroblast of (a) CMC Na 0.5% suspension (b) Cork fish extract (c) Lemon peel extract 400 mg/kgBW (d) Base cream (e) Burnazine cream (f) Lemon peel extract cream 40% (g) Combination of CMC Na 0.5% and Base cream (h) Combination of Burnazine cream and *Hyaluronic acid* (i) Lemon peel extract 400 mg/kgBW suspension and cream 40%.

Figure 3 shows that combined extract at the dose of 400 mg/kg bW and cream at 40% enhanced fibroblast cells and which was comparable with those of combined hyaluronic acid dan krim burnazin.

Based on the research that has been done, this study analyzes the wound-healing activity of the ethanol extract of lemon peel with oral and topical administration of the drug. This study used quantitative methods by measuring the zone of inhibition of antibacterial activity and measuring the diameter of wound healing for 16 days of treatment. The result was in agreement with a previous study conducted by Ahmad et al. [2], which reported that lemon peel extract (*Citrus limon*), Lime (*Citrus paradise*), and Orange (*Citrus sinensis*) given orally at a dose of 400 mg/kgBB for 12 days has therapeutic potential in the treatment of chronic wounds in diabetes. The results showed significant reductions in blood glucose and wound closure time. However, this study has several limitations, we formulated the extract in a conventional dosage form. Further studies are recommended to develop a drug delivery system of cream or suspension formulations of *Citrus limon* extract that can increase the effectiveness of excision wound healing and conduct

further testing with different types of wounds on ethanol extract of lemon peel.

Conclusions

Treatment with a combined oral and topical route (suspension and cream of lemon peel ethanol extract) had better wound healing activity for excision compared to individual oral and topical routes. In addition, lemon peel ethanol extract cream with 40% concentration showed the strongest wound healing activity compared to that of 10% and 20% lemon peel ethanol extract cream. Meanwhile, suspension of ethanol extract of lemon peel at a dose of 400 mg/kgBW displayed the highest wound healing activity compared to those of 100 and 200 mg/kgBW. The antibacterial effect of the extract might contribute to the wound-healing effect of the cream and suspension formulation of the C. limon extract. The results indicate the potency of C. limon extract to be developed into a wound healing agent. However, further studies are required to elucidate their mechanism to enhance the wound healing duration.

Acknowledgements

Authors are very grateful for the research funding assistance that has been provided by TALENTA with contract number 2837/UN5.1.R/PPM/2023, which is under the auspices of Universitas Sumatera Utara, Indonesia, and also BAZNAS Research Scholarship with contract number B/4041/DPPD-DPDS/KETUA/KD.02.18/X/2024.

Declaration of Generative AI in Scientific Writing

The authors acknowledge the use of generative AI tools (e.g., Grammarly, DeepL, and ChatGPT by OpenAI) in the preparation of this manuscript, specifically for language editing and grammar correction. No content generation or data interpretation was performed by AI. The authors take full responsibility for the content and conclusions of this work.

CRediT Author Statement

Risfi Dara Saputri: Conceptualization, Methodology, Supervision, Validation, Funding acquisition, and Writing –original draft.

Yuandani: Conceptualization, Resources, Funding acquisition, Supervision, Validation, and Writing–review & editing.

Aminah Dalimunthe: ConceData curation, Formal analysis, Investigation, Validation, and Visualization.

Abdi Wira Septama: Conceptualization, Data curation, Formal analysis, Investigation, Validation, and Visualization.

Lisda Rimayani Nasution: Data curation, Formal analysis, Investigation, Validation, and Project administration.

References

[1] LF Ang, Y Darwis, RY Koh, KV Gah Leong, MY Yew, LY Por and MF Yam. Wound healing property of curcuminoids as a microcapsule-incorporated cream. *Pharmaceutics* 2019; **11(5)**, 205.

[2] M Ahmad, MN Ansari, A Alam, and TH Khan. Oral dose of citrus peel extracts promotes wound repair in diabetic rats. *Pakistan Journal of Biological Sciences* 2013; **16(20)**, 1086-1094.

[3] H Ansel. *Introduction to Pharmaceutical Dosage Forms*. University of Indonesia Press, Jakarta, Indonesia, 2008.

[4] LV Allen, NG Popovich, and HC Ansel. *Ansel's pharmaceutical dosage forms and drug delivery systems*. 9th ed. Lippincott Williams & Wilkins, Hong Kong, China, 2011.

[5] J Aponno, VY Paulina, Yamlean, and S. Hamidah. 2014, Effectiveness test of gel preparation containing ethanolic extract of guava leaves (*Psidium guajava* Linn) on wound healing infected by *Staphylococcus aureus* in rabbits (*Oryctolagus cuniculus*). Undergraduate Thesis. Sam Ratulangi University, Manado, Indonesia.

[6] D Ariando. *Health analyst pocket book*. 6th ed. Analis Muslim Publish, Bekasi, Indonesia, 2017.

[7] P Bainbridge. Wound healing and the role of fibroblasts. *Journal of Wound Care* 2013; **22(8)**, 407-412.

[8] TF Belachew, S Asrade, M Geta and E Fentahun. *In vivo* evaluation of wound healing and anti-inflammatory activity of 80% methanol crude flower extract of *Hagenia abyssinica* (Bruce) J.F. Gmel in mice. *Evidence Based Complementary*

and Alternative Medicine 2020; **2020(1)**, 9645792.

[9] IH Caesar and BA Dhiani.Formulation and antibacterial activity of fennel fruit (*Foeniculum vulgare* Mill) essential oil lotion. *Media Farmasi* 2014; **11(1)**, 41-54.

[10] MTB Carvalho, HG Araújo-Filho, AS Barreto, LJ Quintans-Júnior, JSS Quintans and RSS Barreto. Wound healing properties of flavonoids: A systematic review highlighting the mechanisms of action. *Phytomedicine* 2021; **90**, 153636.

[11] C Dev and RNS Rishi. Basketful benefit of citrus limon. *International Journal of Pharmaceutics* 2016; **7(6)**, 1-6.

[12] R Elcistia and AK Zulkarnain. Optimization of O/W cream formulation combining oxybenzone and titanium dioxide and in vivo testing of its sunscreen activity. *Majalah Farmasetik* 2018; **14(2)**, 67.

[13] U Fauziah, M Arsyi, Rizki, and YD Safrida. Formulation study and physical stability test of ointment and cream preparations containing ethanol extract of robusta coffee fruit flesh (*Coffea canephora*). *Darussalam Journal of Science and Health* 2022; **2(1)**, 1-9.

[14] A Garg, D Aggarwal, S Garg, and AK Singla. Spreading of semisolid formulations: An update. *Pharmaceutical Technology* 2002; **2002**, 84-102.

[15] H Fitri, A Apriliana, and H Natalia. Characterization and phytochemical screening of Selutui Puka Leaf Simplisia (*Tabernaemontana macracarpa* Jack). *Ibnu Sina Scientific Journal* 2009; **4(1)**, 49-58.

[16] L Hakkinnen, H Larjava and L Koivisto. *Granulation tissue formation and remodelling. In*: H Larjava (Ed.). Oral wound healing cell biology and clinical management. 1st ed. SPi Publisher Services, Pondicherry, Indonesia, 2012, p. 135-139.

[17] P Husni, AN Pratiwi, and A Baitariza. Formulation of a cream containing ethanol extract of moringa leaves (*Moringa oleifera* Lamk). *Jurnal Ilmiah Farmasi Farmasyifa* 2019; **2(2)**, 101-110.

[18] RM Jacob, P Amruth, P Akshay, KVS Rose, R Anandan, and S Mathew. *In vivo* assessment of wound closure and biocompatibility of nanochitosan-polyvinylalcohol-glycerol films in experimental animal model. *International Journal of Zoology and Applied Biosciences* 2023; **8(6)**, 77-83.

[19] T Kurahashi and J Fujii. Roles of antioxidative enzymes in wound healing. *Journal of Developmental Biology* 2025; **3(2)**, 57-70.

[20] K Li, Y Diao, H Zhang, S Wang, Z Zhang, B Yu, S Huang, and H Yang. Tannin extracts from immature fruits of *Terminalia chebula Fructus* Retz. promote cutaneous wound healing in rats. *BMC Complementary and Alternative Medicine* 2011; **11**, 86.

[21] E Lukitaningsih, AH Saputro, M Widiasri, N Khairunnisa, N Prabaswari and R Kuswahyuningsih. *In vitro* anti-aging analysis of a topical pharmaceutical preparation containing a mixture of strawberry fruit, pomelo peel, and langsat fruit extracts. *Indonesian Journal of Chemometrics and Pharmaceutical Analysis* 2020; **1(1)**, 52-60.

[22] N Lumentut, HJ Edi and EM Rumondor. Formulation and physical stability testing of a cream preparation containing 12.5% ethanol extract of Goroho banana peel (*Musa acuminata* L.) as a sunscreen. *Jurnal MIPA* 2020; **9(2)**, 42.

[23] D MacKay and AL Miller. Nutritional support for wound healing. *Alternative Medicine Review* 2003; **8(4)**, 369-370.

[24] NS Majid, VYY Paulina, and C Gayatri. Formulation and antibacterial effectiveness test of jackfruit leaf (*Artocarpus heterophyllus* Lam.) extract cream against *Staphylococcus aureus*. *Pharmacon* 2019; **8(1)**, 225-233.

[25] A Martin and J Swarbick. *Physical pharmacy*. 3rd ed. Lee & Febiger, New York, 1969.

[26] E Middleton, C Kandaswami, and TC Theoharides. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharmacological Reviews* 2000; **52(4)**, 673-751.

[27] EG Molina. Natural bioactive compounds of citrus limon for food and health. *Journal of Pharmaceutical and Biomedical Analysis* 2010; **51(2)**, 327-345.

[28] S Nealma and Nurkholis. Formulation and physical evaluation of cosmetic cream with

variations of Sappan wood extract (*Caesalpinia sappan*) and Sumbawa Beeswax. *Tambora Journal* 2020; **4(2)**, 8-15.

[29] EF Palumpun, AAGP Wiraguna, and W Pangkahila. Topical administration of betel leaf extract (*Piper betle*) increases epidermal thickness, fibroblast count, and collagen count in the wound healing process in male Wistar rats (*Rattus norvegicus*). *e-Biomedic Journal* 2017; **5(1)**, 1-7.

[30] A Paramita. 2016, The effect of Binahong leaf extract ointment (*Anredera cordifolia* (ten) steenis) on collagen density in white rats (*Rattus norvegicus*) with burn wounds. Undergraduate Thesis. Airlangga University, Surabaya, Indonesia.

[31] ACO Silva, EF Santana, AM Saraiva, FN Coutinho, RHA Castro, MNC Pisciottano, ELC Amorim, and UP Albuquerque. Which approach is more effective in the selection of plants with antimicrobial activity? *Evidence-Based Complementary and Alternative Medicine* 2013; **2013(1)**, 308980.

[32] NIMDS Suena, NIPU Antari, and E Cahyaningsih. Physical quality evaluation of body butter formulation from ethanol extract of mangosteen (*Garcinia mangostana* L.) rind. *Indonesian Journal of Pharmaceutical Sciences* 2017; **15(1)**, 63-69.

[33] RE Sucita and IS Hamid. Topical ethanol extract of Sappan wood (*Caesalpinia sappan* L.) is effective on collagen density during the healing phase of incision wounds in white rats. *Journal of Veterinary Medicine* 2019; **2(2)**, 119-126.

[34] IS Suharto and AN Etika. Ginger extract (*Zingiber officinale* Roscoe) affects the collagen fiber density of incision wounds. *Scientific Journal of Health Sciences* 2019; **7(1)**, 27-34.

[35] T Sentat and Permatasari. Activity test of ethanolic extract of avocado leaves (*Persea americana* Mill.) on burn wounds on the backs of male white mice (*Mus musculus*). *Jurnal Ilmiah Manuntung* 2015; **1(2)**, 100-106.

[36] WH Schmitt. *Skin care products. In*: DF Williams and WH Schmitt (Eds.). Cosmetics and toiletries industry. 2nd ed. Blackie Academy and Professional, London, 1996.

[37] R Tuloli, HJ Edi, and I Jayanto. Formulation of a cream preparation combining celery leaf extract (*Apium graveolens* L.) and teak leaf extract (*Tectona grandis* Linn. f) as an antibacterial agent against *Staphylococcus aureus*. *Pharmacon* 2020; **9(2)**, 259.

[38] M Toyoda, H Takayama, N Horiguchi, T Otsuka, T Fukusato, G Merlino, H Takagi, and M Mori. Overexpression of hepatocyte growth factor/scatter factor promotes vascularization and granulation tissue formation *in vitro*. *FEBS Letters* 2001; **509(1)**, 95-100.

[39] W Wahyuni, H Wahid and R Febriana. Formulation of an ethanol extract cream from taro (*Colocasia esculenta* L.) leaf stalks for incised wounds in white rats (*Rattus norvegicus*) of the Wistar strain. *Jurnal Ilmiah Farmasi* 2022; **3(3)**, 338-347.