The Effect Of Binjai Leaves Extract Gel (Mangifera Caesia) And Ramania Leaves Extract Gel (Bouea Macrophylla Griffith.) On The Number Of Fibroblast Cells In Incisional Wound Of Male Rats (Rattus No

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The Effect Of Binjai Leaves Extract Gel (Mangifera Caesia) And Ramania Leaves Extract Gel (Bouea Macrophylla Griffith.) On The Number Of Fibroblast Cells In Incisional Wound Of Male Rats (Rattus Norvegicus)

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1. Introduction

The wound is discontuinty of sructure at epithelium layer of the skin.1 The wound will be followed by wound healing process that consist of 3 phases, called inflammation, proliferation, and remodelling.[2]

During wound healing process, medications such as non-steroid anti-inflammatory drugs (NSAID) will be given to relieve pain and as an antiinflammation.[3] Administration of NSAID may have side effect such as gastric ulcer with anemia.[4] Based on that, people start to use traditional herbal medicine to minimalize the side effect. Traditional herbal medicines that can be found in South Kalimantan are binjai (Mangifera caesia) and ramania (Bouea macrophylla Griffith.)

Binjai and ramania contain secondary metabolites like alkaloids, triterpenoid, flavonoid, saponin, and tannin.[5,6] Flavonoid will work as antioxidant to balance the amount of reactive oxygen species (ROS).[7] Flavonoid can protect the body and support the wound healing process to achieve the proliferation of fibroblast for collagen synthesis.[8,9] Fibroblast begin to active 72 hours after wound occur, then it is increased on the 7th day to start collagen synthesis, and decrease on the 14th day in remodelling stage.[10,11,12]

Binjai extract and ramania extract can be made as topical gel formulation for faster absorption, better deliverance, and afford cool sensation on skin.[13,14]

2. Materials & method

The research process was started through the submission of ethical clearance that was later issued by Ethics Committee of Faculty of Dentistry, University of Lambung Mangkurat No. 075/KEPKG-FKGULM/EC/I/2020. The research method involved true experimental with posttest-only control group design. The sample used was 18 male wistar rat (Rattus norvegicus) with a body weight of 200-250 gram and 2-3 months old which was divided into 3 groups.

The making process of binjai leaves extract used maceration method. A total of 5 kilograms leaves were collected and washed in water. The leaves were dried in the open air then mashed up to obtain simplicia powder. It was macerated for 3 days in 70% ethanol. The result was filtered and concentrated using rotary evaporator at 50°C then it was evaporated using waterbath until it formed a thick ethanol extract. The extract was added with propylene glycol, tween 20, nipagin and nipasol, HPMC, and aquadest.

The method process of ramania leaves extract started with the leaves washed in water, dried using oven at 50°C for 4 hours then mashed up to obtain simplicia powder. The simplicia powder was maceration in 95% ethanol. After 3 days, it was filtered and concentrated using rotary evaporator at 50°C then it was evaporated using waterbath until it formed a thick ethanol extract. The extract added with propylene glycol, tween 20, nipagin and nipasol, HPMC, and aquadest.

The rats were adapted for a week while given regular feed and drink. The rats were anesthetized using a combination injection of ketamine (80 mg/kg)-xylazine (15 mg/kg) peritoneally. The back of rat was cleaned with 70% alcohol and shaved with a size of 3 cm. The wound was made using scalpel no. 15 until the depth of subcutaneous layer.

The topical application of extract gel was performed using cotton bud once daily for 14 days and was later wrapped with gauze. The rats on each group were sacrificed on the 7th day and the 14th day using the combination injection of ketamine (100 mg/kg)-xylazine (20 mg/kg). Afterward, excision for tissue extraction was conducted using scalpel and tissue scissors around 3 cm long and 3 cm width in a depth of subcutaneous layer. The corpse were put in a container and buried within 50 cm depth.

The tissue was fixed in 10% formalin solution, cut to the size of 1 cm length and packed in tissue cassette. Dehydration process was performed using tissue processing for ±18 hours and the tissue was later

taken out. The tissue was transferred into base mold filled by paraffin liquid, then put in embedding cassette until it freeze. Paraffin block were cut off with a thickness of 5 microns. After that, the block was put on waterbath and later fixated on the object glass. Then the specimen was stained using Haematoxylin Eosin (HE). The preparations were observed using microscope with 40x10 magnifications in 5 observing fields.

Statistical analysis used Shapiro-Wilk's normality test and Levene's homogeneity test. Afterward, Two-Way Anova with a confidence level of 95% was followed by Post-Hoc Bonferroni analysis to determine the value of significance.

3. Results

The mean value of fibroblast cells number in incisional wound healing on the back wound of rat in day 7th and day 14th is illustrated in Table 1 and Figure 1.

Table 1. Descriptive Result of Fibroblast Cells Number in Incisional Wound Healing of Rat

Treatment	Day Group	Mean	Standard Deviation
Placebo Gel	Day 7th	10.67 (9-12 cells)	1.53
	Day 14th	7.67 (7-8 cells)	0.58
15% Ramania Gel	Day 7th	16.67 (15-18 cells)	1.16
	Day 14th	12.00 (10-14 cells)	2.00
15% Binjai Gel	Day 7th	16.33 (14-18 cells)	1.53
	Day 14th	11.33 (9-13 cells)	2.08

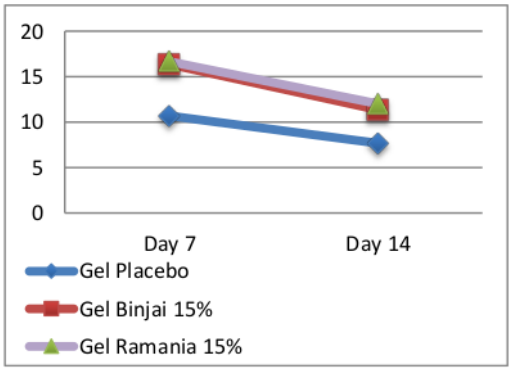

Figure 1. Mean of Fibroblast Cells in Incisional Wound Healing of Rat on Day 7th and Day 14th.

Figure 1 shows the mean of fibroblast cells in incisional back wound of rat in each group on day 7th and day 14th. The highest number of fibroblast cells on day 7th in order is 15% ramania leaves extract gel (16.67±1.16 cells), 15% binjai leaves gel extract (16.33±1.53 cells), and placebo gel (10.67±1.53 cells). On day 14th, mean number of fibroblast cells have decreased. The lowest number of fibroblast cells in order is placebo gel (7.67±0.58 cells), 15% binjai leaves extract gel (11.33±2.08 cells), and 15% ramania leaves extract gel (12.00±2.00 cell).

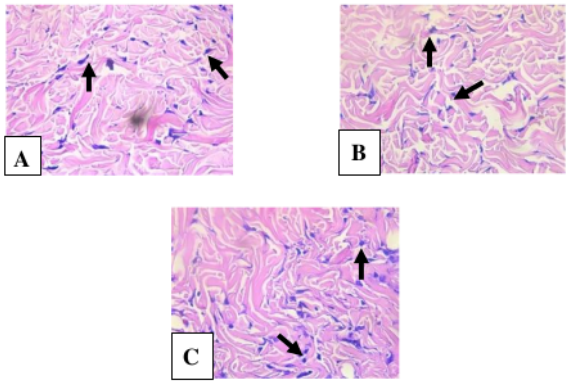

Figure 2. Histopathology of Fibroblast Cells in Incisional Back Wound Healing of Rat on Day 7th: (A) Placebo Gel Group, (B) 15% Binjai Leaves Gel Extract Group, (C) 15% Ramania Leaves Gel Extract Group

Figure 2 shows the histopathology of fibroblast cells number in incisional back wound of rat given placebo gel group with a total of 9-12 cells, binjai leaves extract gel at the concentration of 15% group with a total of 14-18 cells, and lastly ramania leaves extract gel at the concentration of 15% group with a total of 15-18 cells.

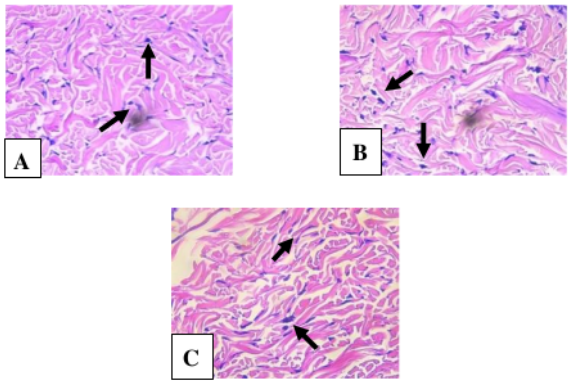

Figure 3. Histopathology of Fibroblast Cells in Incisional Back Wound Healing of Rat on Day 14th: (A) Placebo Gel Group, (B) 15% Binjai Leaves Gel Extract Group, (C) 15% Ramania Leaves Gel Extract Group

Figure 3 shows the histopathology of fibroblast cells number in incisional back wound of rat in placebo gel group with a total of 7-8 cells, binjai leaves extract gel at the concentration of 15% group with a total of 9-13 cells, and lastly ramania leaves extract gel at the concentration of 15% group with a total of 10-14 cells.

Result from statistical analysis shows the data was normally distributed ($p>0.05$) and the distribution of data was homogen ($p>0.05$). Two-way anova test was performed and shows that there is significant difference based on treatments ($p<0.05$) and based on days ($p<0.05$).

Table 2. Two-Way Anova Result Test

Source	*Mean Square*	Sig.
Treatment	48.722	.000
Day	80.222	.000
Treatment * Day	1.722	.514

Table 2 shows the result of Two-Way Anova Test ($p<0.05$). There was a significant difference based on treatments and based on days to the number of fibroblast cells. Meanwhile, the significance value in the interaction between treatment and day was greater than 0.05 which means that there was no significant difference between treatment and day to the number of fibroblast cells. Because there was a significant difference, data analysis was followed by Post-Hoc Bonferroni to find significant difference statistically.

Table 3. Post-Hoc Bonferroni Result Test Based on Treatment

Concentration (I)	Concentration (J)	*Mean Difference* (I-J)	Sig.
Placebo	15% Ramania	-0.517	0.000*
	15% Binjai	-0.467	0.001*
15% Ramania	Placebo	0.517	0.000*
	15% Binjai	0.050	1.000
15% Binjai	Placebo	0.467	0.001*
	15% Ramania	0.050	1.000

Table 3 shows the result that there is significant difference between placebo gel, binjai leaves extract gel at 15% concentration, ramania leaves extract gel at 15% concentration ($p<0.05$). Based on the table, there was no significant difference among binjai leaves extract gel at the concentration of 15% group and ramania leaves extract gel at the concentration of 15% group ($p=1.000$). Based on mean difference between placebo gel group and binjai leaves extract gel at the concentration of 15% group (0.467) and between the placebo gel group and ramania leaves extract at 15% concentration group (0.517), it is revealed that ramania leaves extract gel at the concentration of 15% has better effect compared to binjai leaves extract gel at the concentration of 15%. The result based on day show that there was significant difference between day 7th and day 14th.

4. Discussion

The result of this study proves that binjai leaves extract gel and ramania leaves extract gel was able to increase the number of fibroblast on day 7th and decrease the number of fibroblast on day 14th. Incisional wound in rat will be followed by wound healing process that consist of 3 phase, that is inflammation, proliferation, and remodeling.[2] Fibroblast cell can be found in connective tissue and play an important role to form extracellular matrix (ECM) for wound closure.[15]

Based on figure 1, it is shown the mean number of fibroblast cell on day 7th and day 14th displayed significant difference between placebo gel and treatment groups, such as binjai leaves extract gel at the concentration of 15% and ramania leaves gel extract at the concentration of 15%. This significant difference is due to the flavonoid contained in both leaves.

Flavonoid function as secondary antioxidant to balance the amount of oxidant and antioxidant through hidrogen donor for Nrf2 activation in order to increase the activation of endogen antioxidant, such as superoxidase dismutase (SOD), catalase (CAT), and glutathion peroxidase (GPX).[16,17] Flavonoid will protect

the body so that wound healing process can be proceed and promote the proliferation of fibroblast and collagen synthesis.[7,8] Moreover, flavonoid can work as immunomodulator. Based on research by Kurnia (2015), which used *Camellia sinensis*, flavonoid will support lymphocytes proliferation and interleukin-1 (IL-1) which will stimulate T-cell proliferation and differentiation into T-helper1 (Th1). Th1 will release interferon-γ (IFN- γ) for macrophage activation and produce growth factors to help fibroblast mitogenesis in proliferation phase. Flavonoid plays a role in forming granulation tissue, where fibroblast presents as the predominant cell. Flavonoid will support the increase insulin-like growth factor-1 (IGF-1) as a mediator of fibroblast proliferation. In addition to growth factors, flavonoid can produce cytokines, such as IL-1, IL-4, IL-8 to support fibroblast chemotaxis and keratinocytes.[18,20]

This study result based on figure 1 shows that there is a difference in the mean of fibroblast cell number on day 7th. Ramania leaves extract gel at the concentration of 15% group and binjai leaves extract gel at the concentration of 15% group shows better result compared to placebo gel group. Based on a reseach result by Sabirin (2013) that used *Morinda citrifolia* L. leaves extract in incisional wound of rat, it was obtained that the number of fibroblast cell on incisional wound of rat in day 7th have better result than to control group.

Seven days after wound formation, fibroblast number will achieve their peak and substitute the macrophage in inflammation phase.[21] Macrophage release *growth factors*, such as platelet derived growth factor (PDGF), fibroblast growth factor (FGF), endothelial growth factor (EGF), transforming growth factor-α (TGF-α), and TGF-β, which stimulate proliferation of fibroblast and generate extracellular matrix (ECM) formation.[22] Fibroblast with the help of matrix metalloproteinase (MMP) will seize the fibrin matrix and replace it with glycosaminoglycan (GAG). Extracellular matrix will be replaced by type III collagen which is also produced by fibroblast.[1] Type III collagen is commonly found for tissue repair and achieve its peak on the 5th-7th day after wound formation.[22]

Figure 1 shows that the mean of fibroblast cell on day 14th had decrease. Placebo gel group have number of fibroblast cell that is lower than binjai leaves extract gel at the concentration of 15% and ramania leaves extract gel at the concentration of 15%. According to a research by Ismiardianita et al., (2019), which used *Clausena excavate* extract containing flavonoid in extraction wound of rat, it is contained that the number of fibroblast decreased on day 14th compared to day 7th. After the peak on day 7th, fibroblast will decrease because wound healing process goes well and fibroblast will be more progressive to synthesize the collagen in maturation phase. Another cause of decrease in the number of fibroblast cells is due to phenotype change of fibroblast into myofibroblast. Afterward, deposition of ECM will increase.[12,15,22]

Myofibroblast is derived from fibroblast which has similar characteristics like smooth muscle cells. Myofibroblast contains intracellular actin microfilament and endoplasmic reticulum tissue for matrix protein production. Myofibroblast helps the wound to contract and reconnect.[23] Myofibroblasts are connected by cell-to-cell and cell-to-matrix and express α-Smooth Muscle Action (α-SMA) through repetitive contraction in order to produce collagen fibers in the injury area. [1,22,25] At this stage, type III collagen will be replaced by type I collagen which have band shape and have stronger tensile strength and density in new tissue.[24] Only around 80% tensile strength will be recovered due to collagen fibers ability that can only retrieve 80% of its normal strength before injury.[1]

5. Conclusion

Based on statistical result and discussion show that there is effect of binjai leaves extract gel at concentration of 15% and ramania leaves extract gel at concentration of 15% to the number of fibroblast cell in rat incisional back which will increased on day 7th and decrease on day 14th. Based on statistical result, ramania leaves (*Bouea microphylla* Griffith.) extract gel at concentration of 15% have better result compared to binjai (*Mangifera caesia*) extract gel at concentration of 15% and placebo gel.

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