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THE EFFECT OF BINJAI (*Mangifera caesia*) LEAVES EXTRACT GEL TO COLLAGEN FIBER DENSITY ON WOUND

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A wound is a general experienced by society and it kept increasing every year. The majority of the experienced wound is caused by surgery and trauma, that is 48%.¹ Based on South Kalimantan RISKESDAS (2018), wound caused by the oral surgery procedure reached 8,46% and the tooth extraction is 0,13%.

The main steps of the wound healing process involve inflammation, proliferation, and remodelling. In proliferation phase, fibroblast cell has a role to synthesize collagen that will cover the wound.^{2,3} Collagen becomes the parameter of the tissue forming or skin regeneration.⁴ Collagen is the main protein from extracellular matrix contained on the skin formed by amino acid with triple helix structure.² Hydroxyproline inside the tissue can be used as the parameter index of the collagen amount in the skin. The higher the hydroxyproline content, then it can be indicated that there is an escalating of collagen synthesis that

correlates with the process speed of wound healing.⁴

One of the efforts to help the wound healing process is by giving wound covering gel or antibiotic preparat spreading. The few contents of that medicines can cause side effect onto the skin during the process of wound healing. Herbal treatment starting to widely used by the Indonesian government because it can reduce the side effect compared with the chemical medicines and has more economic value.^{5,6} One of the plants that can be the herbal material is the binjai plant (*Mangifera caesia*) which is a plant that originally from South Kalimantan.

The secondary metabolite compound contents that the binjai leaves extract has are flavonoid, saponin, phenolic, tannin, alkaloid, and triterpenoid.^{7,8} Tannin can reduce the forming of wound tissue caused by strong anti-bacterial and angiogenic activities.⁹ Flavonoid is a strong antioxidant so it can reduce peroxidase lipid, meanwhile alkaloid, saponin and triterpenoid are

potentially being the chemoprotective that can inhibit peroxide lipid.^{7,10} According to Dwidhanti et al (2018), the IC50 value of binjai leaves extract is 2.498,48 µg/mL, so the researcher used binjai leaves extract as one of the adjuvant therapy for wound healing. Adjuvant therapy is an additional therapy that designed for helping to reach the healing process.¹¹ Binjai leaves extract can be a gel formations because it contains a lot of water, so it will be better for the substance penetrates into the tissue.¹²

The researcher wanted to see the effect of binjai leaves (*Mangifera caesia*) extract gel to collagen fiber density on wistar rat (*Rattus norvegicus*) incision wound on the 7th and 14th day. The purpose of this research is to analyze the effect of binjai leaves (*Mangifera caesia*) extract gel that topically given to collagen fiber density on wistar rat (*Rattus norvegicus*) incision wound.

MATERIALS AND METHODS

This research was conducted after the ethical clearance issued by Health Research Ethical Committee Dentistry Faculty ULM with No.072/KEPKG-FKGULM/EC/I/2020. This research used true experimental with post-test only with control group design with random sampling technique consists of 4 groups. Group I as control has been given the placebo gel, group II has been given the 5% concentration of binjai leaves extract, group III with the 10% concentration, and group IV with the 15% concentration. The repetition amount in each treatment were 3 times based on the calculation of numeric analytic formula which > 2 groups are not in pairs. The used sample of this research was the male wistar rat (*Rattus norvegicus*) that has been adapted and fulfill the inclusion criteria. The research was conducted at Basic Laboratory FMIPA Lambung Mangkurat University, Parasitology Laboratory, Jamu Pucuk Sirih Factory Banjarmasin, and Biochemical Laboratory, Medical Faculty, Lambung Mangkurat University.

The Making of Binjai Leaves Extract

The binjai leaves that was taken, then washed and cleaned with water, after that weighed in wet condition as much as 5 kg. The cleaned binjai leaves then dried at the room temperature of 27-30°C without direct sunlight for 4 days. The dried binjai leaves cut into small parts and made into simplicia powder using the blender and 850 g of it was obtained. The simplicia powder shifted first using mesh 12 sieve before conducting the maceration by soaking it using 70% ethanol. The maceration conducted for 3 x 24 hours without direct sunlight, so the solution concentration was equal, then conducted the stirring using a magnetic stirrer with 50 RPM speed for 15 minutes, until the

solvent enters to the whole surface of simplicia powder. The liquid extract was obtained after 72 hours, then concentrated using a rotary evaporator at the temperature 50°C. The filtration result then vaporized in the water bath until the final result was obtained, that is 66,25 g of binjai leaves extract.⁷

The Making of Binjai Leaves Extract Gel

The 100% pure binjai leaves thick extract made into gel formations using 3 different concentrations, that are 5%, 10%, and 15%. The making of the binjai leaves extract gel is by mixing the gel base that contains propylen glycol, Tween 20, nipagin, nipasol, HPMC, and 100% aquades with 5 gr of the binjai leaves extract for 5% concentration, 10 gr of the binjai leaves extract for 10% concentration, and 15 gr of the binjai leaves extract for 15% concentration.¹³

The Animal Experiment Procedures

The experimental animal was adapted by given the food and drink that have been standardized such as BR2 and aquadest. This matter intend to obtain uniformity before conducting the research for experiment animal control.¹⁴ The adapted experimental animal, then prepared to conduct incision injury. Before that, the back fur of the rat shaved with the size 5 cm length and 3 cm height, then conducting anesthesia using 40-100 mg/Kg BW of ketamine and 5-10 mg/Kg BW of xylazine intraperitoneally.¹⁵

The incision wound was made 2 cm and the depth up to subcutis on the right back of Wistar rat aligned with os. vertebrae and 5 cm apart from the ear using sterile blade number 15. The wounded rat then randomly grouped. Binjai leaves extract gel and placebo gel applicated on the wound topically using cotton buds on each experimental animal until it covered the whole wound, then covered using 0,9% NaCl solution dipped gauze.¹⁶

On the 7th and 14th day after the wounding procedure, the euthanasia process was conducted in the rat with giving the mixed anaesthesia agent of 40-100 mg/Kg BW of ketamine and 5-10 mg/Kg BW of xylazine intraperitoneally and wait until the rat is dead. The extraction of tissue on the incision wound area was done by the excisional method with 1 cm x 1 cm length, and until subcutis depth. The experimental animal that has been euthanized, then cleaned and the carcass was wrapped with white fabric then buried with minimal 75 cm depth.¹⁵

The Making of Homogenate

The absorbance of the solution measured at the 550 nm of wave length and hydroxyproline rate was extrapolated using hydroxyproline standard curve that was obtained by using UV-VIS spectrophotometer device with the $y=ax+b$

equation, y is absorbance and x is rated value. The result that was obtained, then analyzed.¹⁵

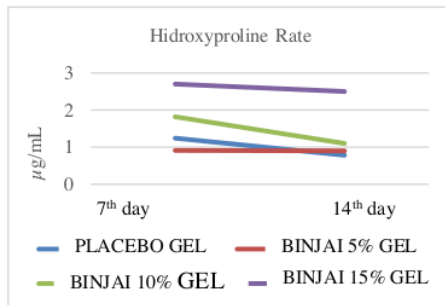
RESULTS

The measuring result of hydroxyproline rate in the wistar rat incision wound can be seen in the table 1.

Table 1. Mean and Standard Deviation the measuring result of hydroxyproline rate in the wistar rat incision wound

Treatment	Mean ± Standard deviation	
	Day-7	Day-14
Placebo	1.238 ± 0.004	0.777 ± 0.004
Binjai 5%	0.915 ± 0.005	0.901 ± 0.007
Binjai 10%	1.822 ± 0.009	1.098 ± 0.008
Binjai 15%	2.701 ± 0.019	2.509 ± 0.102

Table 1 shows that there is a variation difference hydroxyproline rate in the placebo gel group, 5%, 10% and 15% concentrations of binjai leaves extract gel that has been repeated for 3 times.



Picture 1. Diagram mean of hydroxyproline

It can be seen in Picture 1 that the highest hydroxyproline mean is in the 15% concentration of binjai leaves (*Mangifera caesia*) extract gel, then followed by the 10% concentration, the placebo gel and the 5% concentration of binjai leaves (*Mangifera caesia*) extract gel on the 7th day. The amount of hydroxyproline rate in all the groups reduced on the 14th day, with the highest hydroxyproline average rate is the 15% concentration of binjai leaves (*Mangifera caesia*) extract gel, then followed by the 10% concentration, 5% concentration, and the placebo gel.

The average rate of hydroxyproline was normality tested using the Shapiro-Wilk test. Based on the Shapiro-Wilk normality test, it shows that the Sig. value on the 7th day is 0,050 µg/mL and on the 14th day is 0,158 µg/mL. The said significance value ($p > 0,05$) of the research data were stated normally distributed. If the data are normally distribute, then continued with homogeneity test using Levene's test and the obtained value is Sig.p=0.801 ($p > 0,05$). The said value shows that the research data fulfilled the homogeneity requirements. The normally distributed and homogenous data can be parametric analysis tested using the Two-Way Anova with 95% level of confidence. The result of the Two-Way Anova statistic test shows the value $p \text{ value} = 0,000$ ($p < 0,05$) which shows that there is a meaningful difference, then continued with Post Hoc Bonferroni test to find out the group with the most meaningful difference.

The result of the Post Hoc Bonferroni test shows that the value of Sig.p $> 0,05$ in all the groups which means the groups have statistically meaningful difference.

DISCUSSION

Based on this research, it is known that the hydroxyproline rate in all the groups shows the high number in observation of the 7th day. The average of control group that has been given placebo gel shows the number 1,238 µg/mL. The 5% concentration of binjai leaves extract gel treatment group shows smaller number that is 0,915 µg/mL, the 10% concentration is 1,822 µg/mL, and 15% concentration is the highest of all the groups that is 2,701 µg/mL. The high rate of hydroxyproline on the 7th day can be caused by the proliferation phase on that day, which is the early phase of the forming of collagen until collagen synthesis peaked.¹⁷ The hydroxyproline rate amount in the control group is higher than the 5% concentration of binjai leaves extract gel group, but shows the lower hydroxyproline rate than the 10% and 15% concentrations. The said matter can happen because of the secondary metabolite compound content of binjai leaves extract, the higher the gel concentration, the bigger the effect that capable to give for optimizing the wound healing process.

Flavonoid is one of the secondary metabolite compounds that contained in binjai leaves (*Mangifera caesia*) extract, serve as an antioxidant that works in the inflammation phase by increasing endogenous antioxidants which are SOD and CAT.¹⁸ Flavonoid also can arrange migration and proliferation of fibroblast in the area of wound lesion.¹⁹ The other content is triterpenoid

that serve to help the process of reepithelization which is very significant.^{7,20}

Saponin in the binjai leaves can trigger vascular endothelial growth factor (VEGF) and increase the amount of macrophages so it can migrate to the wound area for increasing the production of cytokines that will activate the fibroblast in wound tissue. In the proliferation phase, the fibroblast will produce collagen, therefore the density of collagen fiber on the 7th day observation shows the optimal value.¹⁹

On the 14th day observation, the average rate of hydroxyproline experienced reduction in all groups if compared with the 7th day. The average rate of hydroxyproline on the 14th day in the control group reduced from 1,238 $\mu\text{g/mL}$ down to 0,777 $\mu\text{g/mL}$, the 5% concentration of binjai leaves extract gel shows not so significant reduction from 0,915 $\mu\text{g/mL}$ down to 0,901 $\mu\text{g/mL}$. The average rate of hydroxyproline reduction also happened to the 10% concentration of binjai leaves extract gel from 1,822 $\mu\text{g/mL}$ down to 1,098 $\mu\text{g/mL}$, and the 15% concentration from 2,701 $\mu\text{g/mL}$ down to 2,509 $\mu\text{g/mL}$. This can happen because on that day the wound healing in wistar rat had already in remodelling phase, resulting in the balance of synthesis and degradation of collagen with the help of the collagenase enzyme that works by absorbing the excessive collagen.²¹ The stable density of collagen fiber on the 14th day caused by tannin in the binjai leaves (*Mangifera caesia*) extract. Tannin serves to stabilize the collagen and also can reduce the forming of wound tissue caused by strong anti-bacterial and angiogenic activities.⁹

Binjai leaves extract gel application with 3 different concentrations shows a meaningful difference with the control group. The hydroxyproline rate in the binjai leaves extract gel application, the 15% concentration shows the highest rate amounts that are 2,701 $\mu\text{g/mL}$ on the 7th day and 2,509 $\mu\text{g/mL}$ on the 14th day compared with the 5% and 10% concentrations and placebo gel. In all three concentrations, it can be seen that the hydroxyproline rate is getting higher every 5% increased and optimal in 15% concentration on the 7th day, then stable on the 14th day with the rate still optimal in 15% concentration.

Based on the statistic data result and the discussion, it shows that there is an effect of binjai leaves extract gel (*Mangifera caesia*) to collagen fiber density on wistar rat (*Rattus norvegicus*) incision wound. In the treatment groups that have been given binjai leaves extract gel, the hydroxyproline rate is higher than groups that have been given the placebo gel as the control group because the binjai leaves (*Mangifera caesia*)

extract contains a few active compounds that can help the effectiveness of skin's wound healing. This research concludes that the 15% concentration of binjai leaves extract gel has the most optimal effect than the 5% and 10% concentrations of to collagen fiber density on wistar rat (*Rattus norvegicus*) incision wound.

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