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Research Article

The Potential Activity of Kecombrang (*Etlingera elatior*) Leaf, Flower, and Stem Extracts on the Duration of Wound Healing in Mice (*Mus musculus)*

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ABSTRACT

Wound is skin tissue damage due to trauma from a sharp object. Kecombrang flower extract is known to be effective in speeding up the healing of wounds because it contains flavonoids, saponins, tannins and steroids. The leaves and stems also contain these compounds, so this research was conducted to see the difference in the potential activity of the wound healing process of the kecombrang plant on the time of wound healing. Natural ingredient extracts are wound healing agents that can fight infection and speed up wound healing. This study used 5 treatment groups consisting of control (-) distilled water, control (+) Povidone Iodine 10 %, leaf extract 10 %, flower extract 10 %, and stem extract 10 %. The parameters observed were the length of the wound and the condition of the wound until the wound healed. The obtained data were analyzed statistically using ANOVA for complately randomized factorial designed. The results of the research show that extracts of kecombrang leaves, flowers, and stems have activity in accelerating the healing process of cut wounds and produce different effects on healing time. Wound healing time for leaf extract is 12 days, flower extract and stem extract is 10 days. The conclusion of this study shows that there is a very real difference in wound healing from treatment with the best treatment for wound healing is the flower extract with highest healing score of 6.42.

Keywords: Flower extract**;** Healing process; Leaf extract; Stem extract; Wound

INTRODUCTION

The skin is the largest organ of the body and performs the same function throughout its structure. Skin has many functions, including protection, thermoregulation, and moisture regulation. Physiologically, the body can repair damaged skin tissue automatically when body tissue is injured or injured. This response takes the form of cell regeneration and wound healing to restore the structure and function of damaged body tissue (Ferdinandez et al., 2013). According to Puspitasari et al. (2016), wounds are damage to skin tissue caused by sharp objects such as knives, razors, glass, sharp axes, or swords.

People generally use Povidone Iodine 10 % as initial treatment if minor injuries occur. However, apart from its effect as an antiseptic, Povidone Iodine is also toxic to fibroblasts (Danarti et al., 2014). Therefore, other alternative medicines are needed for wound treatment (Castelain et al., 2016). The use of natural medicines as a substitute for chemical medicines has been widely used because they have advantages, such as: being easier to obtain, low price, and safe from sensitivity reactions (Sutadipura et al., 2015).

The ability to heal wounds cannot be separated from the content of active compounds in natural ingredients, such as alkaloids, saponins, tannins, and flavonoids (Hasibuan et al., 2015). One plant that

contains these metabolites is the kecombrang plant (*Etlingera elatior*). Kecombrang is one of the spice plants in Indonesia which is widely used traditionally as a flavoring ingredient in food in several countries (Suryani et al., 2019).

Based on previous research conducted by Sagala et al. (2016), 96 % ethanol extract of kecombrang flowers (*Etlingera elatior*) at a concentration of 5 % showing faster time to heal wound in white rats (*Rattus novergicus*) that is 10 days compared to Povidone Iodine 10 % which has a healing time of 11 days. Nonci et al. (2016), reported that 96 % ethanol extract of kecombrang leaves with concentrations of 1.5 % and 2 % was able to inhibit *Pseudomonas aeruginosa* bacteria. Meanwhile, research by Azizah and Samodra (2022) showed that ethyl acetate extract ointment from kecombrang stems had inhibitory activity against *P. aeruginosa* at a concentration of 10 %. *Pseudomonas aeruginosa* known as a pathogenic bacteria in humans and can infect wounds which characterized by the formation of pus (Prihandani, 2015; Suhartati & Nurasiah, 2016).

The ability of kecombrang to heal wounds cannot be separated from the active compounds contained in these natural ingredients, such as alkaloids, saponins, tannins, and flavonoids (Hasibuan et al., 2015). The 96 % ethanol extract of kecombrang flowers contains flavonoids, saponins, tannins, and steroids (Pulungan, 2018), meanwhile Jabbar et al. (2019) reported that 96 % ethanol extract of kecombrang leaves and stems contains alkaloids, flavonoids, tannins, and saponins. The presence of these metabolite compounds plays an important role in biochemical reactions as a promoter of proliferation during the formation of fibroblasts and collagen (Risa et al., 2018). Kecombrang flower extract has the potential to heal wounds faster than povidone iodine.

The kecombrang has a comparatively uniform compound content in its leaves, flowers, and stems. Therefore, this study was carried out using leaf, flower, and stem extracts from the kecombrang plant to look for possible changes in wound heal activity on mice (*Mus musculus*) on the certain period of time. Mice were prefered as test animal rather than rat considering they are more similar metabolism and physiological features to human (Ridwan, 2013).

METHODS

Instrumen and Materials

The instrumens used include : Shaver, alcohol swab, Mesh sieve no. 40, blender, desiccator, caliper, batis cloth, mouse cage, filter paper, porcelain crucible, tray, oven, stirrer, dropper, knife, scalpel, rotary evaporator, spatula, 1 mL syringe, test tube, thermometer, scale (Ohaus®), vials, and maceration containers. Material used : aquades, ammonia, anhydrous acetic acid (Merck), hydrochloric acid (Merck), iron (III) chloride. Kecombrang stems, flowers and leaves of Kecombrang from Sukabumi area, West Java, 70 % ethanol, 96 % ethanol, magnesium powder, concentrated H_2SO4 (Merck), ketamine (Ethica), male white mice, methanol, mouse food (pellets 512), Dragendorf's reagent (Sigma Aldrich), Mayer's reagent (Sigma Aldrich), povidone iodine 10 % (Mahakam Beta Farma).

Material Collection, Determination, and Research Ethics

The leaves, flowers, and stems of kecombrang (*Etlingera elatior*) were obtained from Sukabumi Regency, West Java. Plant determination was carried out in the Herbarium Depokensis (UIDEP), Biota Collection, University of Indonesia. The research has obtained ethical permission no. 013/KEPHP-UNPAK/07-2023 for the Use of Experimental Animals from the Ethics Committee FMIPA, UNPAK.

Preparation of Kecombrang Dry Powder and Extract

The leaves, flowers, and stems of kecombrang (*Etlingera elatior*) were wet sorted, washed, cut into smaller pieces then dried in the sun covered with a black cloth. Dry kecombrang was powdered using a blender then sieved using Mesh sieve no. 40, the dry powder furthermore was stored for extraction process.

About 500 g, 300 g, and 1000 g of kecombrang leaf, flowers, and stems were respectively put in a maceration container and added with 96 % ethanol in a ratio of (1: 10). The maceration container was stored in a place protected from sunlight. Then it was stir occasionally during the first 6 hours of soaking, then leave for 18 hours. The soaking results are filtered to separate the dregs and filtrate, the dregs are macerated again with 96 % ethanol at half the volume of solvent in the first filtering and left again for 3 × 24 hours then filtered. The filtrate is collected and concentrated using a rotary evaporator to obtain a condensed extract.

Phytochemical Screening

Tests include flavonoid test, alkaloid test, saponin test, tannin test, and triterpenoid/steroid test on each leaf, flower, and stem extract of kecombrang. To test the presence of flavonoid compounds, 0.5 g leaf, kecombrang leaf, flower and stem extract dissolved in 3 mL of methanol, then evaporated until dry. The dry extract was put in a test tube and added with 2-3 drops of ethanol, Mg powder, and a few drops of 5 mol hydrochloric acid. Positive results indicating the presence of flavonoids are characterized by the appearance of a red to violet color (Hanani, 2015).

The alkaloid test was carried out by weighing 1 gram of each kecombrang extracts and mixing it with 3 mL of ammonia and 20 mL of methanol. The

mixture was heated for 15 minutes at 60 °C while shaking. About 3 mL of solution was taken and added with 5 mL of 1N hydrochloric acid was added. An orange precipitate indicated the presence of alkaloids when the Dragendorff's reagent was added and the appearance of a white precipitate showed the presence of alkaloids when the Mayer's reagent was applied to the second tube (Hanani, 2015).

The saponin test was carried out by adding 10 mL of water to each 0.5 gram of kecombrang extract. Then hydrochloric acid was added to the solution. The production of stable foam indicated the presence of saponin. To test the presence of tannin compounds, 0.2 g of each extract of combrang leaves, flowers and stems was dissolved in 10 mL of boiling water. The blue to blackish green color that appears after adding a 3% iron (III) chloride solution indicated the presence of tannin (Hanani 2015).

The tritepenoid/steroid test was carried out by dissolving 0.1 g each of kecombrang leaf, flower and stem extract in ether. Then 5 drops of anhydrous acetic acid and 3 drops of concentrated sulfuric acid were added. If a red or purple color appears, it indicated the presence of triterpenoid compounds in the sample, while the presence of steroids compounds were indicated by the presence of a green or blue color (Dwisari et al., 2016).

Preparation and Maintenance of Mice

The mice used were male mice (*Mus musculus*) aged 2-3 months with body weight of 20-30 g. The mice used were healthy, moved actively, and had no anatomical abnormalities. To ensure the homogeneity of the mice body weight, each was weighed and the Coefficient of Variation (CV) in mice body weight must be under 15 %. The total number of mice used was 25, divided into 5 treatment groups, each group containing 5 mice. The size of the cage for each group is 40×30×18 cm. The test animals underwent acclimatization for one week before treatment. During acclimatization, the mice were given ®512 pellets containing 22 % protein and unlimited access to tap water.

Testing for Cut Wounds

Mice were anesthetized with ketamine at a dose of 0.08 mg/gBW intramuscularly (i.m.). An incision was made on the left side of the back with a length of 1.5 cm and a depth of 0.2 cm using a sterile scalpel. The blood that comes out is wiped with an alcohol swab. After that, 0.1 mL of the test sample was applied to the wound for 5 mice in each treatment group twice a day, in the morning and evening at the same time (Table 1).

Wound Score Criteria

Observations of the wound score were carried out every day until the wound healed. The criteria observed were the length of the wound and the condition of the wound. The length of the wound was measured using a caliper, and the condition of the wound was scored. The wound score criteria are as follows: Score 1. Red wounds, severe edema, wet, open wounds; 2. Red wounds, mild edema, wet, open wounds; 3. Pale red wound, slightly dry wound edges, narrowed wound; 4. The edges of the wound are slightly dry, and the wound is narrowed; 5. The wound is narrowed, the edges of the wound are hard, and the scab has formed; 6. The wound becomes narrower and shallower, the scar becomes soft, scab forms; 7. Visible scab residue, soft scars, narrowed wounds; 8. The wound has closed, and the scab is no longer there.

Data Analysis

The obtained data was satatistically analyze using ANOVA for completely randomized design (CRD) with 5 treatment factors and the number of observation days to determine any differences between treatments.

Determination of Water and Ash Content

The water content of kecombrang dry powder extracs were determined using gravimetry methods. About 2 g of each the extracts from the leaves, flowers, and stems of the kecombrang (*Etlingera elatior*) were put in a porcelain crucible. Dried at 105 ºC for 3 hours and weighed. Drying was continued and weighed 1 hour apart until the difference between 2 consecutive weighings was no more than 0.25 % (Wahyuni, 2021).

Table 1. Treatment Group of Mice

Group	Treatment
Control (-)	Mice were given distilled water without extract
Control (+)	Mice were given Povidone iodine 10 %
Leaf	Mice were given leaf extract kecombrang 10 %
Flower	Mice were given flower extract kecombrang 10 %
Stem	Mice were given stem extract kecombrang 10 %

Determination of Ash Content

The ash content of kecombrang dry powder extracts were determined using gravimetry methods. About 2 g of each the extracts from the leaves, flowers, and stems of the kecombrang were put in a porcelain crucible that has been ignited and weighed. Then it was ignited slowly by gradually increasing the temperature to 600 °C. Then the crucible was cooled and weighed again until a constant weight was obtained, that was, the difference was no more than 0.25 % (Fadhila et al., 2022).

RESULTS AND DISCUSSION

Yield of Kecombrang Extracts

Determination of kecombrang was carried out to ensure the type of plant used. The determination confirm that the sample used was *Etlingera elatior* (Jack) R.M.Sm. from the Zingiberaceae family. The dry powder obtained from the leaves, flowers, and stems of kecombrang were presented in Table 2.

Tabel 2. The Dry Powder Yielded from Leaf, Flower, and Stem of Kecombrang

Parts of plant	Raw material (g)	Dry Powder (g)	Yield (%)
Leaf	5.000	648.73	12.97
Flower	3.000	319.5	10.65
Stem	8.000	1.884	23.55

The yield dry powder from leaves was 12.97 %, flowers 10.65 %, and stems 23.55 %. The purpose of making powder is to reduce the particle size. The small particle size will expand contact with the filter, making it easier to penetrate the cell walls and making the compounds in dry powder more easily dissolved (Diniatik, 2015).

The extract was made using the maceration method. Extracts from the leaves, flowers, and stems of kecombrang were obtained by extracting the active substances from dry powder using 96 % ethanol solvent. The yield of the extract obtained is shown in Table 3.

The yield of extract obtained from leaves was 4.57 %, flowers were 8.48 %, and stems were 1.58 %. This yield was greater than research conducted by Jabbar et al. (2019), that are 4.37 % and 1.5 % for leaves and stems respectively. The yield of flower extract was 8.48 % which greater the than the yield of Rahmiyani et al., research (2023) that is 7.85%. The yield of extracts is related to the amount of compounds contained in it (Dewatisari et al., 2018).

Phytochemical Screening

Phytochemical screening of kecombrang leaf extract contains flavonoids, alkaloids, saponins, and tannins. Meanwhile, extracts of kecombrang flowers and stems contain flavonoids, alkaloids, saponins, tannins, and steroids. The results of phytochemical screening are shown in Table 4.

The compounds in the extract were obtained extracting the leaf, flower, and stem of kecombrang plant using 96 % ethanol as solvent. The 9 6% ethanol was used as a solvent because its high ability to attract non-polar, semi-polar, and polar compounds in the plant sample (Wendersteyt et al., 2021).

Table 3. The Yield of Kecombrang Leaf, Flower, and Stem Extract

Part of Plant	Simpliscia weight (g)	Extract weight (g)	Yield %
Leaf	500	22.89	4.57
Flower	300	25.46	8.48
Stem	1.000	15.84	1.58

Table 4. Phytochemical Screening of Kecombrang Leaf, Flower, and Stem Extracts

Extract	Flavonoid	Alkaloid	Saponin	Tanin	Triterpenoid	Steroid
Leaf	+	+	+	+	-	-
Flower	+	+	+	+	-	+
Stem	+	+	+	+	-	+

Table 5. Extract Water and Ash Content of Kecombrang Extracts

Extract	Water content (%) ± SD	Ash content (%) ± SD
Leaf	9.26 ± 0.22	3.84 ± 0.53
Flower	7.80 ± 0.33	3.33 ± 0.53
Stem	8.14 ± 0,04	5.19 ± 0.53

Preparation and Maintenance of mice

The mice were first acclimatized for one week prior to the treatment to accustom mice to their environment. Th CV of mice body weight was 7.62 %, before acclimatization and 6.57 % after acclimatization. The results confirmed that the body weight of mice was homogeneous with CV value under 15 % (Nasution, 1992).

Water and Ash Content of Kecombrang Extracts

Extracts of kecombrang leaves, flowers, and stems were checked for water and ash content. This test aims to determine the water and ash content contained in the extract.

The water content of the extract meets the establish quality requirements ≤ 10 % standard (Kementrian Kesehatan RI, 2017). Water content is related to the storage time of extract, the high water content in extract (> 10 %) will facilitate and increase bacterial growth, resulted in reducing the stability of the extract (Diniatik, 2015).

The ash content of kecombrang leaf, flower, and stem extracts aims to determine the mineral content from the initial process to the formation of the extract (Kementrian Kesehatan RI, 2017). The test results for the ash content of leaf extract were 3.84 %, flower extract 3.33 %, and stem extract 5.19 %. These results meet the ash content requirements that is no more than 3.9 % to 17.4 % (Ministry of Health of the Republic of Indonesia, 2017). The value of ash content indicates the presence of mineral and also heavy metal contamination that is resistant to high temperatures (Ratnani, 2015).

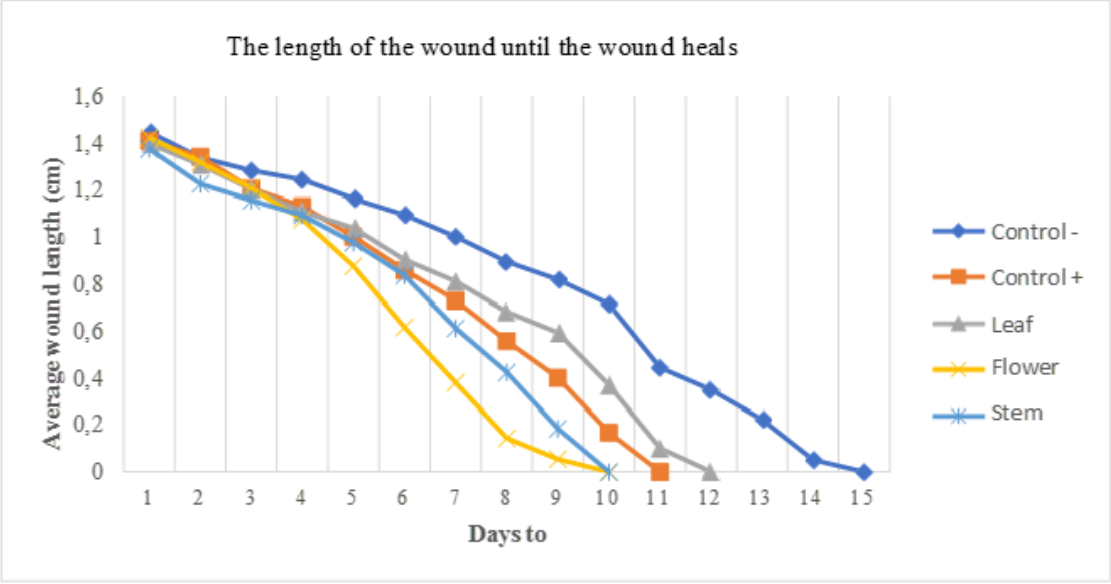

Figure 1. Average of wound length until the wound was heal

Observation of Wound Healing

Mice were anesthetized with ketamine at a dose of 0.08 mg/g BW before the incision to reduce pain during the wounding process. Observation of wound healing was observed by measuring the reduction of wound length. Measurements were carried out every day using a caliper.

Based on observation results, kecombrang flower and stem extract had a faster wound healing time on the day 10 compared to the Povidone Iodine 10 % (+ control) on day 11. meanwhile the kecombrang leaf extract showed slower wound healing on the day 12. The longest wound healing was shown by the control (-) where the wound was healed on the 15th day. The flower extract has the best effect in healing wounds healing time which was similar to the research of Sagala et al. (2016) where the10 % concentration of kecombrang flower extract showed shorter time in healing wounds on rats (Rattus novergicus) on the day 10.

The healing effect on wound treated with extracts of leaves, flowers, and stems of kecombrang extracts cannot be separated from the compounds contained in plant such as flavonoids, alkaloids, saponins, tannins, and steroids. The compound contained in extracts of kecombrang leaves, flowers, and stems is useful in accelerating wound healing. Flavonoid compounds have antimicrobial and astringent activity (Barku et al., 2013). Tannins and steroids are known to have anti-inflammatory activity (Meilina et al., 2018; Hertian & Muhaimin, 2021). Saponins and alkaloids act as antibacterials and saponins have the ability to stimulate the production of type I collagen which plays a role in increasing tissue epithelialization (Larissa et al., 2017; Haryati et al., 2015). Leaf extracts have a longer wound healing time compared to flower and stem extracts, probably caused by the lack of steroid content in the leaves as confirmed in the results of phytochemical screening. Steroids have an anti-inflammatory effect by inhibiting enzymes that play a role in the synthesis of arachidonic acid which then produces inflammatory mediators (Hertian & Muhaimin, 2021). The observation mice wound healing was carried out by measuring the parameter of injury scoring method as seen in Table 6.

Table 6. The Average of Injury Score in Mice During Kecombrang Treatment

Day to Day Observations	Average Wounds Score					
	Control -	Control +	Leaf	Flower	Stem	X ± SD
1	$1,4^{a}$ ± SD	$1,8^{ab}$ ± SD	$1,6^{ab}$	$1,6^{ab}$	$1,8^{ab}$	$1,64^{a}$ ± 0,16
2	$2,2^{bc}$	$2,8^{cde}$	$2,6^{cd}$	$2,8^{cde}$	3^{def}	$2,68^{b}$ ± 0,30
3	$3,2^{defg}$	$3,8^{gh}$	$3,4^{efg}$	$3,6^{fg}$	$3,8^{gh}$	$3,56^{c}$ ± 0,26
4	$3,8^{gh}$	$4,8^{ijk}$	$4,4^{hi}$	$5,2^{jkl}$	5^{ijkl}	$4,64^{d}$ ± 0,55
5	$4,6^{ij}$	$5,4^{klm}$	$5,2^{jkl}$	$6,2^{nop}$	$5,6^{lmn}$	$5,52^{e}$ ± 0,59
6	$5,6^{lmn}$	6^{mno}	6^{mno}	$6,6^{opqr}$	$6,2^{nop}$	$6,08^{f}$ ± 0,36
7	6^{mno}	$6,2^{nop}$	$6,2^{nop}$	7^{qrst}	$6,6^{opqr}$	$6,40^{g}$ ± 0,40
8	$6,2^{nop}$	7^{qrst}	$6,6^{opqr}$	$7,6^{tuv}$	7^{qrst}	$6,88^{h}$ ± 0,52
9	$6,4^{opq}$	7^{qrst}	7^{qrst}	$7,8^{uv}$	$7,4^{stuv}$	$7,12^{i}$ ± 0,52
10	$6,8^{pqrs}$	$7,4^{stuv}$	$7,2^{rstu}$	8^{v}	8^{v}	$7,48^{j}$ ± 0,52
11	7^{qrst}	8^{v}	$7,6^{tuv}$	8^{v}	8^{v}	$7,72^{k}$ ± 0,43
12	7^{qrst}	8^{v}	8^{v}	8^{v}	8^{v}	$7,8^{kl}$ ± 0,44
13	$7,2^{rstu}$	8^{v}	8^{v}	8^{v}	8^{v}	$7,84^{kl}$ ± 0,35
14	$7,8^{uv}$	8^{v}	8^{v}	8^{v}	8^{v}	$7,96^{l}$ ± 0,08
15	8^{v}	8^{v}	8^{v}	8^{v}	8^{v}	8^{l} ± 0
X ± SD	$5,54^{a}$ ± 2,05	$6,14^{c}$ ± 2,03	$5,98^{b}$ ± 2,10	$6,42^{e}$ ± 2,14	$6,29^{d}$ ± 2,04	

Note: Numbers followed by the same superscript letter indicate no significant difference between treatments in wound healing in mice ($\alpha<0.05$) based on Duncan's advanced test. The higher wound score indicates the best wound healing.

Results of statistical analysis show there are significant differences between treatments and length of wound healing time (days). The differences depicted in the difference score which located in same column, but has different superscript letters. Kecombrang flower extract had the highest healing effect on cut wounds with an average score of 6.42, followed by stem extract with a value of 6.29, control (+) 6.14, leaf extract 5.98, and control (-) 5.54. The score changes every day according to the treatment given. Wound conditions improve over time where open wound narrows and closes. All treatments showed no significant differences in wound scores after day 15 except for the control (-) where the wound healing occurred on day 15. There was an interaction between treatment and healing time (days) which affected the condition of the cut wound over time.

The wound healing process generally has 3 stages starting from the inflammatory phase, proliferation phase, and remodeling phase (Arisanty, 2013). Inflammatory phase (initial wound appears until day 3 or 5), proliferation phase (from day 2 to day 24), and remodeling phase (from day 24 to 1 or 2 years) (Arisanty, 2013). The healing process in mice related to the phytochemical compounds contained in the extracts of kecombrang leaves, flowers, and stems which play a role in the inflammatory and the proliferation phase. A score of 1–3 indicates that the wound is in the inflammatory phase and a score of 4–8 indicates that the wound is in the proliferative phase.

The process that occurs in the inflammatory phase, the wound cause the damage of blood vessels and bleeding condition. The body tries to stop the bleeding with vasoconstriction, shrinking the tip of the broken blood vessels, and hemostasis reactions. The platelets that have left the blood vessels stick together and form a fibrin network to clot the blood that comes out of the blood vessels (Sjamsuhidajat, 2017). The proliferation phase consists of a destructive process (cleaning phase), a proliferation or granulation process (release/growth of new cells), and epithelialization (cell closure/migration) (Arisanty, 2013). During the destructive phase, there is the cleaning of dead tissue (which experiences devitalization) and bacteria carried out by polymorphs and macrophages (Sjamsuhidajat, 2017). Polymorphs and macrophages also stimulate the formation of fibroblasts. When the wound surface is closed, the fibroblast process with the formation of granulation tissue also stops and the maturation process (Sjamsuhidajat, 2017).

CONCLUSION

Kecombrang leaf, flower, and stem extracts have activity in accelerating the wound healing process with a healing time of 12 days for the leaves extract, 10 days for the flowers extract, and and10 days for the stems extract. Statistical analysis showed that there are significant effects in each treatment on the wound healing process from initial open wounds to healed closed wounds. The conclusion of this study state that the best treatment for wound healing is the flower extract with highest healing score of 6.42.

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