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Cover Page Footnote

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ORIGINAL ARTICLE

Effects of Lime (*Citrus aurantifolia Swingle*) Peel Extract Addition to Periodontal Dressing on the Number of Blood Vessels in the Gingival Wound Healing Process

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ABSTRACT

Background: Periodontal dressings that are currently available can only protect wounds from mechanical trauma, but have no ability to accelerate wound healing. Lime (*Citrus aurantifolia Swingle*) peel extract added to periodontal dressing contains flavonoids which can stimulate the formation of new blood vessels, thus accelerating the healing process. **Objective**: To determine the effects of adding lime (*Citrus aurantifolia Swingle*) peel extract to periodontal dressing on the number of blood vessels in the gingival wound healing process. **Methods:** 32 rabbits with 2 mm wound made on the gingiva were divided into 8 groups. For the control groups of K1(n=4) and K2(n=4) periodontal dressings were applied without the addition of lime peel extract. Meanwhile, the treatment groups P1(n=4) and P4(n=4), P2 (n=4) and P5(n=4), and P3(n=4) and P6(n=4), 5%, 10%, and 15% lime peel extract, respectively, were added to the periodontal dressing. Groups K1, P1, P2, and P3 were scarified after 3 days, whereas groups K2, P4, P5, and P6 were scarified after 5 days. Histological observations of tissue by Hematoxylin Eosin (HE) staining were carried out to count the number of blood vessels. **Result:** The results of the statistical tests show that group P5 had the highest number of new blood vessels. **Conclusion:** The addition of lime peel extract to periodontal dressing can increase the number of blood vessels in the gingival wound healing process.

Key words: blood vessels, lime (*Citrus aurantifolia Swingle*) peel extract, periodontal dressing

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INTRODUCTION

To cure periodontal diseases and minimize the number of bacteria present, procedures in periodontal surgery that require a wide field of view are indispensable. Therefore, incisions is needed that causes open wounds.[1] In general, open wounds are then protected by periodontal dressings,[2] whose main function is to protect wounds from mechanical trauma and stabilize the surgical site during the healing process. Currently, the existing periodontal dressings do not have a curative function and contain no ingredients that can improve the wound healing process or stimulate healing factors. Consequently, additional materials that can help the wound healing process are extremely necessary.[3]

The composition of periodontal dressing has changed over the years. At present, the constituent materials can be broadly divided into three categories as follows: (1) those containing zinc oxide and eugenol, (2) those with zinc oxide but without eugenol, and (3) those without both zinc oxide and eugenol.[4]

Eugenol has antibacterial and analgesic effects, but may cause hypersensitivity and allergic reactions.[5] Periodontal dressings containing zinc oxide but without eugenol are known to inhibit wound healing, which is characterized by inflammation even after seven days of application. One of the periodontal dressings containing neither zinc oxide nor eugenol is the cellulose-based periodontal dressing, which is non-toxic to cells so it does not hinder the wound healing process.[1]

Physiologically, human body has a mechanism for repairing damaged tissues by forming new structures, which is known as the wound healing process.[8]

Wounds can be defined as loss of skin continuity. The wound healing process consists of several phases, namely homeostasis, inflammation, migration, proliferation, and maturation.[9] In the proliferation phase, angiogenesis or the formation of new blood vessels occurs.[10]

Angiogenesis is one of the indicators of successful wound healing.[11] New blood vessels have a vital role in transporting nutrients and oxygen needed for the activities of fibroblasts and epithelial cells. These new blood vessels will increase the number of fibroblasts in the wound area to meet nutritional needs as well as produce plasminogen activator and collagenase so that the wound can heal faster. Furthermore, adequate tissue formation is followed by migration and proliferation of decreased endothelial cells and cell death.[10]

The development of science has led to the discovery of numerous herbal ingredients that can be added to periodontal dressings so as to accelerate wound healing, one of which is lime (*Citrus aurantifolia Swingle*) peel that can treat inflammation in postoperative wounds. Lime is incredibly beneficial for the health of the body, especially the oral cavity. In fact, lime peel alone has natural antioxidant, antibacterial, and anti-inflammatory properties.[12]

Lime peel, as well as its leaves and flesh, contain flavonoids that inhibit inflammation.[12] Flavonoids also stimulate macrophages to release cytokines and growth factors such as VEGF and FGF-2 in the inflammatory and proliferative phases. These growth factors increase angiogenesis or the formation of new blood vessels.

Lime peel also contains quercetin, a flavonoid compound that can stimulate Hypoxia Inducible Factor-1 (HIF-1), which then induces Vascular Endothelial Growth Factor (VEGF), thereby accelerating the process of angiogenesis.[6] Meanwhile, saponins in lime peel can stimulate angiogenesis by increasing the production of VEGF.[7]

The flavonoid content in lime (*Citrus aurantifolia Swingle*) peel makes it suitable for use as an added ingredient to periodontal dressings so as to accelerate wound healing. In a previous study, lime peel extract is proven to increase the number of blood vessels on the third and fifth days during the healing process of rat post tooth extraction socket.[13] Thus, this present study aims to find out whether a periodontal dressing containing lime (*Citrus aurantifolia Swingle*) peel extract has an effect on the number of blood vessels in the gingival wound healing process.

METHODS

This in vivo laboratory experimental study applied the Posttest-Only Control Group Design.

Research sample

This study was approved by the Animal Care and Use Committee of Universitas Brawijaya Malang, with Ethical Clearance number: 051-KEP-UB- 2021. 32 samples were divided into 8 groups. The control groups (K1, K2) were given periodontal dressing without lime peel extract. Meanwhile, lime peel extract was added to the periodontal dressing applied to the treatment groups, with a percentage of 5% for groups P1 and P4, 10% for groups P2 and P5, and 15% for groups P3 and P6. The inclusion criteria for the sample were male New Zealand white rabbit, healthy, aged 4-5 months, weighed 3-4 kg, without previous treatment or exposure, and not disabled, whereas the exclusion criteria were rabbits that died during the study.

Research period

This study was carried out for ± 4 months in the Stem Cell Laboratory of Universitas Airlangga.

Research procedure

The experimental animals were selected according to the sample criteria and then adapted for 7 days. The production of lime peel extract was followed by maceration method using 70% ethanol solvent, resulting in a thick extract.

Baer's formulation of periodontal dressing consists of powder and paste. First of all, powder dressing was made by mixing 28.5 g of rosin and 21.5 g of zinc oxide on a paper stirrer using a GIC spatula until homogeneous. Separately, paste dressing was made by mixing 47.5g of hydrogenated fat and 2.5g of zinc oxide until well-combined on a paper stirrer using a GIC spatula. After that, 50 mg of powder and 50 mg of paste were mixed gradually until homogeneous to obtain 100 mg of periodontal dressing for each group.[14] Then, the lime peel extract was added to the periodontal dressings.

After the periodontal dressings were ready, surgical procedures were performed on the experimental animals. 70% alcohol was used to disinfect the surgical site. The animals were anesthetized using a combination of ketamine (25 mg/kg of body weight) and xylazine (3 mg/kg of body weight) intramuscularly. Each rabbit underwent surgery on the lower incisor area of the attached labial gingival mucosa with the same control treatment, namely: (1) using a punch biopsy tool with a diameter of 2 mm, and (2) pressing the wound to reach the alveolar bone but not to the point of damaging it. Then, the wound was cleaned with a solution of 0.9 ml NaCl and 3% H_2O_2.

Follow up was done by applying a periodontal dressing to the wound area. The periodontal dressing was made in accordance with the shape of the wound. After application, the periodontal dressing was gently pressed using a dental excavator to cover the gingival wound area. To increase the retention of the periodontal

Table 1. Baer's formulation of periodontal dressing with or without lime peel extract

Group		Baer's Periodontal Dressing Formula (mg)	Lime Peel Extract (mg)
Day-3	K1 0%	100	0
	P1 5%	95	5
	P2 10%	90	10
	P3 15%	85	15
Day-5	K2 0%	100	0
	P4 5%	95	5
	P5 10%	90	10
	P6 15%	85	15

dressing, sutures were made between the lip and the lower gingival layer using a 5.0 floss thread.

Sampling was done on the 3rd and 5th day to examine the number of blood vessels in the gingival granulation tissue of each experimental group. The experimental animals were scarified under anesthesia using a lethal dose of ketamine, i.e., 200 mg / kg BW, by intramuscular injection. Then, the gingival granulation tissue was placed in a container with 10% formalin solution. The tissue preparations used hematoxylin-eosin staining. Observation of blood vessels was made using an Olympus digital optical microscope at 400x magnification from 5 different fields of view. The Shapiro-Wilk statistical test and Levene's test were performed to prove that the data were homogeneous and normal. Furthermore, the analysis was continued with one-way ANOVA test, Tukey's post-hoc test, and independent T-test.

RESULTS

The number of blood vessels in the treatment group is found to be higher than that of the control group (See Figure 1). The addition of lime (*Citrus aurantifolia Swingle*) peel extract to the treatment group has made the wound healing process take place faster.

Observations on blood vessels with Hematoxylin-Eosin (HE) staining obtained results as shown in Figures 1 and 2 below:

The results of the calculations of the average numbers of blood vessels in the control groups (K1 and K2) and the treatment groups (P1, P2, P3, P4, P5, and P6) are presented in Figure 3.

Based on the graph above, the average number of new blood vessels on Day 3 of the gingival wound healing process experienced an increase in the treatment groups with 5% and 10% lime peel extract, and a decrease

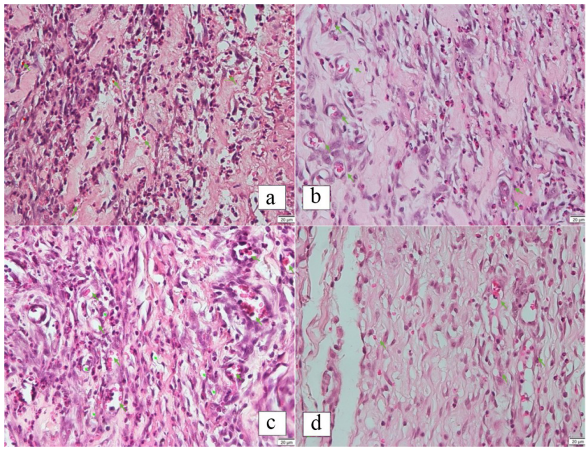

Figure 1. [Day 3] Blood vessels with HE staining, magnification 400x. a. (K1) periodontal dressing without lime peel extract, b. (P1) periodontal dressing with 5% lime peel extract, c. (P2) periodontal dressing with 10% lime peel extract, d. (P3) periodontal dressing with 15% lime peel extract.

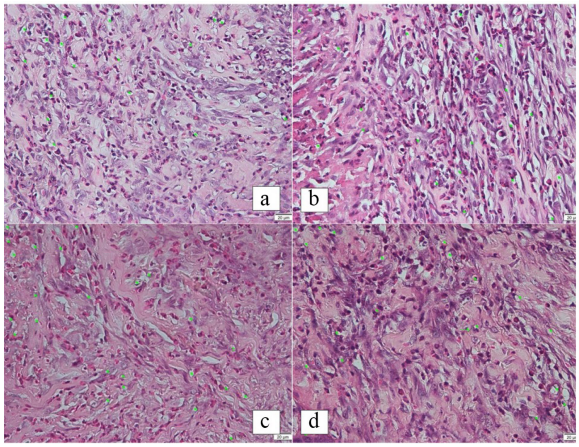

Figure 2. [Day 5] Blood vessels with HE staining, magnification 400x. a. (K2) periodontal dressing without lime peel extract, b. (P4) periodontal dressing with 5% lime peel extract, c. (P5) periodontal dressing with 10% lime peel extract, d. (P6) periodontal dressing with 15% lime peel extract.

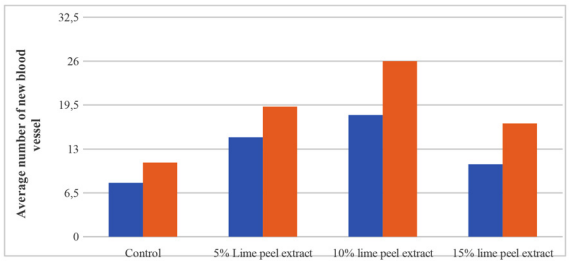

Figure 3. The average numbers of new blood vessels

in the treatment group with 15% lime peel extract. Similarly, the same trend was observed on Day 5. Overall, the addition of 10% lime peel extract resulted in the highest average number of blood vessels on Day 3 and Day 5, compared to the other groups.

Table 2. One-Way ANOVA test results on blood vessels

Observation	Group	Mean	Sig.
Day-3	K1	8.00	0.000
	P1	14.75	
	P2	18.00	
	P3	10.75	
Day-5	K2	11.00	0.000
	P4	19.25	
	P5	26.00	
	P6	16.75	

Table 3. The Results of Tukey's HSD Post-Hoc Test on Day 3

Group	Sig.
K1 – P1	0.003
K1 – P2	0.000
K1 – P3	0.023
P1 – P2	0.008
P1 – P3	0.002
P2 – P3	0.008

Table 4. The Results of Tukey's HSD Post-Hoc Test on Day 5

Group	Sig.
K2 – P4	0.000
K2 – P5	0.000
K2 – P6	0.000
P4 – P5	0.000
P4 – P6	0.016
P5 – P6	0.000

Observations on 32 rabbits divided into 8 groups were made by counting the number of blood vessels in histology sample preparations with 400x magnification. The obtained results of the blood vessel calculations were then processed using the One-way ANOVA test. Before undergoing the One-way ANOVA test, the Shapiro-Wilk normality test and the T-test ($p>0.05$) were done previously to show that the research data is normally distributed.

The One-Way ANOVA test on Days 3 and 5 obtained Sig 0.000 (sig <0.05), which indicates the effect of adding lime (*Citrus aurantifolia Swingle*) peel extract to periodontal dressing on the increase in the number of blood vessels in the gingival wound healing process on Day 3 and Day 5.

The Tukey's Post-Hoc tests were carried out separately to see the pure effect of each treatment day. The results indicated differences in the number of blood vessels in the gingival wound healing process on Day 3 and Day 5, with each group showing a significant difference

Table 5. Independent T-Test

Group	Observation	Mean	Sig.
5% Lime Peel Extract	Day 3	14.75	0.000
	Day 5	19.25	
10% Lime Peel Extract	Day 3	18.00	0.000
	Day 5	26.00	
15% Lime Peel Extract	Day 3	10.75	0.000
	Day 5	16.75	

(sig < 0.05). This proves that the addition of lime (*Citrus aurantifolia Swingle*) peel extract to periodontal dressing is able to significantly increase the number of blood vessels in the gingival wound healing process on Day 5, with a concentration of 10% resulting in the highest increase, followed by concentrations of 5% and 15%.

To determine whether there is a difference in the number of blood vessels in the gingival healing process between observation days, an independent t-test was conducted. The results showed a significant increase in the number of blood vessels ($p <0.05$) from Day 3 to Day 5 in all treatment groups.

DISCUSSION

Based on the calculations of blood vessels on Day 3 and Day 5, the highest number was found in the treatment groups that were given periodontal dressings with the addition of 10% lime peel extract (P2 and P5), followed by the treatment groups with 5% lime peel extract (P1 and P4) and those with 15% lime peel extract (P3 and P6). Meanwhile, the control groups given periodontal dressing without the addition of lime peel extract (K1 and K2) showed the lowest number of blood vessels. The highest average number of blood vessels in the gingival wound healing process was seen in the treatment group with 10% lime peel extract added to the periodontal dressing on Day 5 (P5), whereas the lowest average number of blood vessels was in the control group given periodontal dressing without lime peel extract on Day 3 (K1). This is suspected to be due to the content of active substances in the 10% lime peel extract added to the periodontal dressing formula, which can optimally increase the number of blood vessels. The ability of active substances in accelerating wound healing is not always in line with the increase in their percentage, as not all active substances given in higher concentrations can give better results.[15]

The results of the One-Way ANOVA test found that there were significant differences between the treatment groups ($p<0.05$). This shows that flavonoids, the active substances in lime peel, can help the process

of blood vessel formulation (angiogenesis) during the proliferative phase of the gingival wound healing process.[10]

Neovascularization and granulation tissues are stimulated by growth factors such as PDGF, TGF- β, and VEGF produced by macrophages. Flavonoids have been shown to increase TGF-β which further increases VEGF, a major molecule in vascular growth. Flavonoids contained in lime peel extract have a proangiogenic effect by increasing VEGF expression, thereby accelerating the process of angiogenesis and wound healing.[10]

From the histological observations of the post-injury gingival granulation tissue, it is known that new blood vessels are formed on Day 3 and increased on Day 5. This is in accordance with the statement of Nugroho et al. that the formation of new blood vessels begins on the 3rd to the 7th day, which is during the proliferative phase.[16] At this stage, new capillaries will reach the microvascular network to form granulation tissue, which is new connective tissue formed during wound healing process.[17] Blood vessels will mediate and monitor the exchange of small molecules and limit the transport of macromolecules in order to meet nutritional needs for nutrients for wound healing.[18] After the granulation tissue has been formed, the process of angiogenesis will stop and the formation of blood vessels will decrease, signifying that the wound has completely healed.[19]

On both Day 3 and Day 5 after injury, the number of blood vessels in the control group was less than that of the treatment groups. Each group also had a significant difference ($p < 0.05$) on both days according to the Tukey's Post-Hoc test.

The lowest average number of blood vessels was found in the control groups (K1 and K2). However, the number of blood vessels in both groups continued to increase, proving that angiogenesis can occur naturally in response to wound healing.[20]

In the treatment groups, the increase in the average number of blood vessels is also promoted by the contents of the added lime peel extract. Flavonoids have antibacterial and anti-inflammatory properties, while alkaloids in lime peel are able to disrupt the integrity of the peptidoglycan constituent of bacteria, and eventually killing them. Saponins, on the other hand, show antibacterial potential and are effective in preventing infection in wounds. In addition, lime peel also contains tannins, which act as antioxidant with antibacterial activity. These antibacterial substances contained in lime peel will protect the wound from infection, thereby accelerating the wound healing process.[21]

A previous study by Krismaya et al. that tested the phytochemical substances in lime peel extract found that the highest content was saponins at a concentration of 3.05%, followed by flavonoids at 2.78%, tannins at 2.14%, and then alkaloids at 1.86%.[13] This shows that the most dominant active substances with antibacterial and anti-inflammatory activities in lime peel are saponins and flavonoids.

The addition of lime peel extract at concentrations of 5% and 10%, the results were homogeneous and the periodontal dressing was perfectly applied. Meanwhile, the addition of 15% lime peel extract achieved less homogeneous result, and the periodontal dressing became too sticky and wet so that it could not be placed perfectly onto the wound. In addition, it is also possible that the addition of active substances in high concentrations is not good for wound healing. For example, high amount of quercetin contained in flavonoids will have a toxic effect on wounds.[15] The addition of 5% lime peel extract obtained less optimal result compared to the addition of lime peel extract at a concentration of 10%. This is probably because the active substances contained in lime peel do not work optimally at a concentration of 5%.

A study conducted by Yuliani et al. on the gingival wounds of rats covered with periodontal dressing with 10% *kepel* leaf extract found an increase in the number of new blood vessels, with the average number of blood vessels on Day 5 being 30.[22] Meanwhile, the present study found the average number of blood vessels of 26 on Day 5 in the group with 10% lime peel extract added to the periodontal dressing that was applied on rabbit's gingiva wound. Despite using different experimental animals and additional ingredients, both studies indicated that a concentration of 10% is effective in accelerating wound healing. In addition, another prior study by Naba'atin et al. on the addition of cocoa pod extract to periodontal dressing also discovered the effectiveness of a concentration of 10% in increasing the number of fibroblasts.[15]

CONCLUSION

The number of blood vessels is higher in the treatment group than in the control group. There is a relationship between the addition of lime (*Citrus aurantifolia Swingle*) peel extract and the number of blood vessels in the healing process of gingival wounds. Thus, the addition of lime (*Citrus aurantifolia Swingle*) peel extract to periodontal dressing has an effect on increasing the number of blood vessels in the gingiva wound healing process.

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CONFLICT OF INTEREST

There is no conflict of interest in this study.

REFERENCES

1. Budisidharta Y, Syaify A, Lastianny SP. The effects of zinc oxide non-eugenol and cellulose as periodontal dressings on open wounds after periodontal surgery. Dent J (Majalah Kedokteran Gigi). 2020; 53(1):45-9.
2. Rajeswari G, Murugan M. GC-MS analysis of bioactive components of *Hugonia mystax L*. (Linaceae). Res J Pharm Biol Chem Sci. 2013; 29(17):818-24.
3. Takei N, Carranza K. Clinical periodontology. 11th ed. St. Louis: Mosby Elsevier;2019.
4. Baghani Z, Kadkhodazadeh M. Periodontal dressing: A review article. J Dent Res Dent Clin Dent Prospects. 2013; 7(4):183-91.
5. Faiga NN, Rachmadi P, Meizarini A. Neovascular pattern in wound healing after zinc oxide and curcuma longa rhizome extract dressing application. Contemp Clin Dent. 2018; 9(Suppl 2):S337-41.
6. Jeon H, Kim H, Choi D, Kim D, Park SY, Kim YJ, Kim YM, Jung Y. Quercetin activates an angiogenic pathway, hypoxia inducible factor (HIF)-1-vascular endothelial growth factor, by inhibiting HIF-prolyl hydroxylase: a structural analysis of quercetin for inhibiting HIF-prolyl hydroxylase. Mol Pharmacol. 2007; 71(6): 1676-84.
7. Majewska I, Gendaszewska-Darmach E. Proangiogenic activity of plant extracts in accelerating wound healing - A new face of old phytomedicines. Acta Biochim Pol. 2011; 58(4):449-60.
8. Sumbayak EM. Tinjauan pustaka fibroblas: Struktur dan peranannya dalam penyembuhan luka (Fibroblasts literature review: Their structure and role in wound healing). Jurnal Kedokteran Meditek. 2015; 21(57):1169.
9. Handi P, Sriwidodo, Ratnawulan S. Review sistematik: Proses penyembuhan dan perawatan luka (Systematic review: Wound healing andtreatment process). Farmaka. 2017; 15(2):251-6.
10. Carvalho MTB, Araújo-Filho HG, Barreto AS, Quintans-Júnior LJ, Quintans JSS, Barreto RSS. Wound healing properties of flavonoids: A systematic review highlighting the mechanisms of action. Phytomedicine. 2021; 90:153636.
11. Mustafida RY, Munawir A, Dewi R. Efek antiangiogenik ekstrak etanol buah mahkota dewa *(Phaleria macrocarpa (Scheff.) Boerl.)* pada membran korio alantois *(CAM)* embrio ayam (Antiangiogenic effects of *mahkota dewa* fruit (*Phaleria macrocarpa* (Scheff.) Boerl.) ethanol extract on the chorioallantoic membrane (CAM) of chicken embryo). e-Jurnal Pustaka Kesehatan. 2014; 2(1):5–9.
12. Adindaputri Z, Purwanti N, Wahyudi IA. Pengaruh ekstrak kulit jeruk nipis *(Citrus aurantifolia Swingle)* konsentrasi 10% terhadap aktivitas enzim glukosiltransferase Streptococcus mutans (Effect of lime (*Citrus aurantifolia* Swingle) peel extract with concentration of 10% on the activity of *Streptococcus mutans*-derived glucosyltransferase enzyme). Majalah Kedokteran Gigi Indonesia. 2013; 20(2):126.
13. Krismaya N, Pramudya R, Sati PYI, Kamadjaja DB. Effects of lime (*Citrus aurantifolia* christm. swingle) peel extract on fibroblast proliferation and angiogenesis in rat's tooth extraction sockets. Biochem Cell Arch. 2019; 19(December 2019):4917-9.
14. Pradita AU, Dhartono AP, Ramadhany CA, Taqwim A. Periodontal dressing-containing green tea *Epigallocathechin gallate* increases fibroblasts number in gingival artifical wound model. J Dent Indones. 2013; 20(3):68-72.
15. Naba'atin I, Wahyukundari MA, Harmono H. Penambahan ekstrak kulit buah kakao *(Theobroma cacao L.)* pada periodontal dressing terhadap kepadatan kolagen luka gingiva kelinci (Addition of cocoa (*Theobroma cacao* L.) peel extract to periodontal dressing on collagen density of rabbit gingival wounds). Indones Dent Student J. 2015; 3(2):28-38.
16. Nugroho AM, Elfiah U, Normasari R. Pengaruh gel ekstrak dan serbuk mentimun *(Cucumis sativus)* terhadap angiogenesis pada penyembuhan luka bakar derajat IIB pada tikus wist*ar* (Effect of cucumber (*Cucumis sativus*) extract gel and powder on angiogenesis in healing grade IIB burns in wistar rats). e-Jurnal Pustaka Kesehatan. 2016; 4(3):443-8.
17. Pratama AR, Wathoni N, Rusdiana T. Peranan faktor pertumbuhan terhadap penyembuhan luka diabetes: Review (The role of growth factors in diabetic wound healing: A review). Farmaka. 2017;1 5(2):43-53.

18. Fatmawati F, Wijaksono W. Tumor angiogenesis. Jurnal Respirasi. 2018; 4(3):102-9.
19. Wijayanti CDW, Sudiana IK, Nugraha J. Potensi ekstrak Jatropha multifida terhadap ekspresi VEGF aphthous ulcer Rat norvegicus (Potency of *Jatropha multifida* extract against VEGF expression in aphthous ulcer in *Rat norvegicus*). Jurnal SainHealth. 2017; 1(2):5-12.
20. Larjava H. Oral wound healing: current state and future challenges. [Editorial]. 2013;1–3.
21. Rahman FA, Haniastuti T, Utami TW. Skrining fitokimia dan aktivitas antibakteri ekstrak etanol daun sirsak *(Annona muricata L.)* pada Streptococcus mutans ATCC 35668 (Phytochemical screening and antibacterial activity of soursop (*Annona muricata* L.) leaf ethanol extract on *Streptococcus mutans* ATCC 35668). Majalah Kedokteran Gigi Indonesia. 2017; 3(1):1.
22. Yuliani AS. Pengaruh penambahan ekstrak daun kepel *(Stelechocarpus burahol)* 10% pada periodontal dressing terhadap angiogenesis dalam proses penyembuhan luka gingiva (Effect of 10% *kepel* (*Stelechocarpus burahol*) leaf extract addition to periodontal dressing on angiogenesis in the process of gingival wound healing) [bachelor's dissertation]. Yogyakarta: Universitas Gadjah Mada; 2018.

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