

The effect of 3% binahong leaf extract gel on the wound healing process of post tooth extraction

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ABSTRACT

Background: One of the common frequently performed procedures in dentistry is tooth extraction. After tooth extraction, a series of biological events occurs in the alveolar socket that ultimately results in healing of the socket. Binahong (*Anredera cordifolia*) is an herbaceous plant that contains active compounds that may accelerate the wound healing process. **Purpose:** The aim of this study is to investigate the effect of 3% binahong leaf extract gel on the healing process of the postextraction wound. **Methods:** 3% binahong leaf extract gel was prepared with the composition of 1 g carbopol, 1 g hydroxypropylmethylcellulose (HPMC), 4 g glycerin, 3 g triethanolamine (TEA), 0.04 g Nipagin, 0.04 g Nipazol, 1.2 g binahong leaf extract and Aq ad 40 g. A total of 18 people were subjected to tooth extraction and were then divided into two groups, with nine people in the binahong group and nine in the control group. Following that, residual socket volume (RSV) was examined on days 3, 7 and 14 after the extraction in both the binahong and control groups. The RSV value was obtained by calculating the mesiodistal x buccolingual width x the depth probing and measured using a calliper and probe. Repeated analysis of variance (ANOVA) and Friedman test followed by Wilcoxon and dependent t-test ($p < 0.05$) were used to analyse data. **Results:** Significant difference in the mean RSV between the control and binahong groups was observed on days 3, 7 and 14 post tooth extraction. The RSV value in the binahong group was lower when compared to the control group ($p < 0.05$). **Conclusion:** 3% binahong leaf extract gel can accelerate the process of healing socket wounds.

Keywords: 3% binahong extract gel; binahong; residual socket volume; socket healing; tooth extraction

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INTRODUCTION

One of the more frequent procedures in dentistry is tooth extraction.¹ After tooth extraction, a series of biological events occurs in the alveolar socket that ultimately results in socket healing. The series of biological events that occur during healing are vascular changes; inflammation; cells migration, proliferation and differentiation; maturation of the extracellular matrix and bone formation. These biological events combine to restore the lost tissue.¹

When the tooth is extracted, in the empty socket remains the cortical bone (lamina dura), which is covered by the torn periodontal ligament, with a rim of gingiva epithelium left at the coronal portion.³ Blood will fill the socket then coagulate to seal the socket from the oral cavity. During the first week

of healing, inflammation occurs.^{3–5} Contaminated bacteria and debris left in the socket are destroyed by white blood cells.³ The growth of fibroblasts and capillaries indicates the onset of fibroplasia in the first week of healing. Under the blood clot, the epithelium migrates down the socket wall until it comes into contact with the epithelium on the other side of the wall socket or encounters the granulation tissue. There is accumulation of osteoclasts along the crest bone during the first week of healing.^{3,4}

An abundance of granulation tissue fills the socket by the second week.⁵ There has been osteoid deposition along the alveolar socket bone. In the smaller socket, the epithelium may be completely intact by this time.^{3,4,6}

The process that started during the second week continues into the third and fourth weeks of healing.

By this time, the epithelialization of most of the socket has already finished.^{3,5} Cortical bone resorption in crest and socket walls continues, and there has been deposition of new trabecular bone.^{3,4,6} The cortical socket bone is completely resorbed in less than four to six months. On radiographs, it is characterised by the absence of the lamina dura. As the bone fills the socket, the epithelium moves towards the cristae and becomes aligned with the adjacent gingival crest. After a year, fibrous tissue or scar is the only remaining visible sign at the alveolar margins of the edentulous area.^{3,6}

The use of medication can reduce the chance of complications and accelerate the postextraction wound healing process. To reduce the inflammatory process and postoperative pain, oral surgeons usually prescribe non-steroidal anti-inflammatory drugs (NSAIDs), but the intake of NSAIDs has several adverse effects, mainly gastrointestinal and haematological problems, renal alterations and mucous and skin reactions.^{7,8} Due to their minimal adverse effects, herbal ingredients are starting to be used as a substitute for chemical drugs.

Anredera cordifolia, commonly known as binahong, is a herbal plant and is believed to bring various health benefits due to its active compound.⁹ Binahong leaf contains flavonoids, terpenoids, oleanolic acid, ascorbic acid, tannins and saponins.¹⁰ As an anti-inflammatory, flavonoids inhibit the metabolic pathway of arachidonic acid, prostaglandin formation and histamine release in inflammation so that in this phase, macrophages can easily carry out their function as phagocytes for debris cells and microorganisms present in the wound.^{11,12} The antimicrobial effect is obtained from triterpenoids and saponins. To inhibit microbial growth, terpenoids interfere with membrane and cell wall formation so that the membrane or cell wall is not completely formed or is formed but incomplete. Saponins can interfere with the permeability of the bacterial cell membrane, which results in the destruction of cell membrane, causing the release of proteins, nucleic acids and nucleotides, which are important components of bacteria. Saponins also have the ability to act as an antiseptic, which kills or suppresses the growth of microorganisms in the wound to prevent more severe infection.¹¹ According to Hanafiah et al.,¹³ 3% binahong leaf extract gel promotes better wound healing in palatal mucosa than 5% and 7% binahong leaf extract gel.

Based on the above description, the researchers are interested in identifying the effect of 3% binahong leaf extract gel in accelerating the healing process of soft and hard tissues in postextraction wounds in humans. The main objective of this study is to investigate the effect of 3% binahong leaf extract gel on the postextraction wound healing process.

MATERIALS AND METHODS

The study type was clinical experimental with posttest study design with a control group. This study was carried

out at the Oral and Dental Hospital, Universitas Sumatera Utara between June and July 2020. The Research Ethics Commission Universitas Sumatera Utara approved this study (No. 55/KEP/USU/2020), according to the Declaration of Helsinki on medical protocols and ethics.

The population used in this study were all oral surgery patients at Oral and Dental Hospital, Universitas Sumatera Utara who had teeth extracted corresponding with inclusion and exclusion criteria. The inclusion criteria were patients aged 20–30 years, not currently taking drugs, no history of systemic disease that could affect wound healing, undergoing extraction of lower molar without complications and generally good oral hygiene. The exclusion criteria were patients who were not willing to be treated and patients who withdrew during the follow-up period.

The sampling technique in this study was simple random sampling in which samples that corresponded with inclusion and exclusion criteria were taken randomly from a population. The sample size in this study was calculated using the sample size formula for hypothesis test of the mean difference between two unpaired groups, referring to a study conducted by Yüce et al.¹⁴ The number of samples in this study was 18 people, consisting of nine control groups and nine treatment groups.

The binahong leaf came from a family medicinal plant in Simpang Pergendangan Village, Tiga Binanga Village, Karo Regency, North Sumatra Province. The selected binahong leaves were those that were completely opened and at least 12 weeks old. The planting did not use pesticides. The binahong leaves that were collected were washed under running water, drained, then weighed as wet weight. This material was then dried in a drying cabinet at a temperature of $\pm 35^{\circ}\text{C}$, until the consistency of the binahong leaf was dry and dark brown. It was then extracted with 70% ethanol. Furthermore, the 3% binahong leaf extract gel was made with the composition of 1 g carbopol, 1 g hydroxypropylmethylcellulose (HPMC), 4 g glycerin, 3 g triethanolamine (TEA), 0.04 g Nipagin, 0.04 g Nipazol, 1.2g binahong leaf extract and Aq ad 40. g.¹³

Subjects that corresponded to the inclusion criteria were asked to sign a written informed consent form to participate in this study after understanding the aims and procedures of the study and their benefits and rights as a subjects. All subjects received full mouth scaling before the tooth extraction, so oral hygiene was optimal. The tooth extraction was carried out at the Oral and Dental Hospital, Universitas Sumatera Utara. Before the tooth extraction procedure, patients' vital signs were checked by the operator. Subsequently, patients were instructed to rinse their mouths with a 0.2% chlorhexidine solution as an asepsis measure. To extract the lower first molar, the operator performed mandibular block anaesthesia and submucous infiltration with articaine 4% (Septanest) 1:100,000 in the region of the tooth to be extracted. After the clinical signs of successful anaesthesia were observed, the operator began an extraction procedure, including loosening of the gingiva using a raspatorium, loosening

and lifting of the tooth from the socket using an elevator, extracting the tooth using forceps and grinding the sharp bone using a bone file. After extraction was complete, the socket was irrigated with sterile saline.¹⁵ In the treatment group, 0.3 cc binahong leaf extract gel was smeared directly in the post-extraction socket, once a day, after brushing teeth at night before sleep, for 14 days, while in the control group, no gel application was done. Patients were given postextraction instructions, such as to bite the tampon for 30–60 minutes, not to consume hot food and drinks, not to rinse too vigorously, not to play with the postextraction wound area with the tongue and not to suck the postextraction wound area.^{16,17}

Clinical evaluation of wound healing was performed by measuring the residual socket volume (RSV). RSV is the ratio of the socket volume. The RSV value was obtained by calculating the mesiodistal width x bucolingual width x the probing depth. Mesiodistal and bucolingual width was measured with a calliper. Probing depth was measured with a UNC-15 probe (Figure 1).¹⁸ RSV examination was carried out on the 3rd, 7th and 14th day after extraction.

The measurement results were recorded, and the data were processed statistically to determine the differences between the two groups. The Shapiro-Wilk test was used to examine the data's normality. Data was presented in tabular form and processed using IBM SPSS Statistics for Windows, version 25.0 (New York, USA). The data were

analysed using repeated analysis of variance (ANOVA) to identify the differences in each group and the Friedman test followed by Wilcoxon's and a dependent t-test to identify the differences between groups ($p < 0.05$).

RESULTS

Based on the clinical examination of the postextraction socket, it was shown that the application of 3% binahong leaf extract gel did not cause any irritation or inflammation symptoms. The clinical conditions of the postextraction socket in the binahong group were better when compared to the control group, in terms of the colour and consistency of surrounding tissue and bleeding, as shown in Figure 2.

In the control group, the RSV mean decreased at each measurement interval. In the control group, the highest mean RSV value was on the 3rd day of examination, and the lowest was on the 14th day of examination. The results of repeated ANOVA tests showed that there was a significant change in the size of the RSV in the control group ($p = 0.001$). The results of a paired wise comparison test showed a significant difference in the RSV for each measurement ($p < 0.05$) (Figure 3).

In the binahong group, the mean of RSV decreased at each measurement interval. Similarly to the control group,

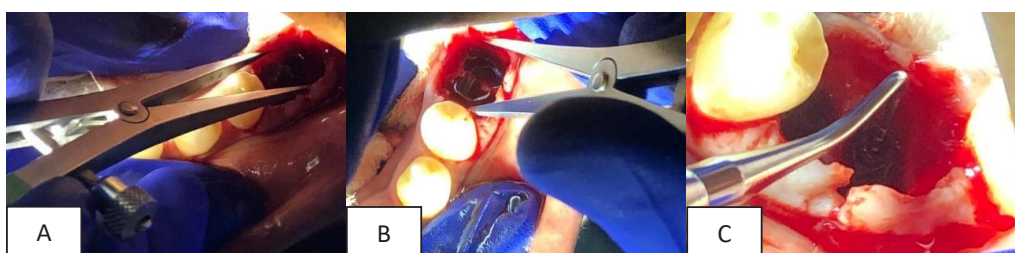


Figure 1. RSV measurement (A) bucolingual width, (B) mesiodistal width, and (C) probing depth.



Figure 2. Clinical picture of post-extraction socket groups (A) control and (B) binahong.

the highest mean RSV value in the binahong group was found on the 3rd day of examination, and the lowest was on the 14th day. The post hoc result using a Wilcoxon test (for data that were not normally distributed) and a dependent t-test (for data that were normally distributed) showed a significant difference in RSV for each measurement. Those were between the 3rd and 7th day ($p = 0.008$), the 3rd and 14th day ($p = 0.008$), and the 7th and 14th day ($p = 0.004$) (Figure 4).

Observed at each measurement interval, the mean of RSV in the binahong group was lower than the control (Figure 5). The results of the Mann-Whitney test showed that there was a significant difference in the mean of RSV between the control and binahong groups on the 3rd day after extraction. The results of the dependent t-test showed a significant difference in the mean of RSV between the control and binahong groups on the 7th day ($p = 0.001$) and 14th day ($p = 0.015$) after extraction.

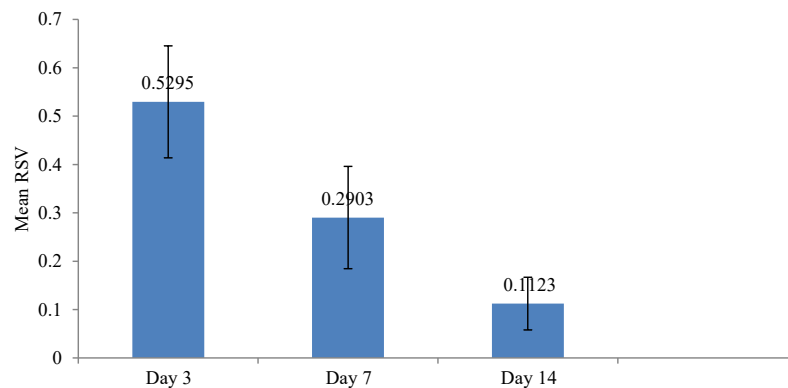


Figure 3. Repeated ANOVA test result RSV in control group.

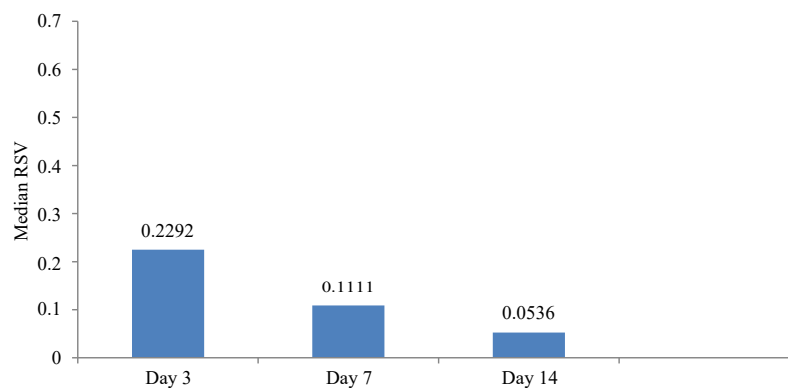


Figure 4. Friedman test result RSV in the binahong group.

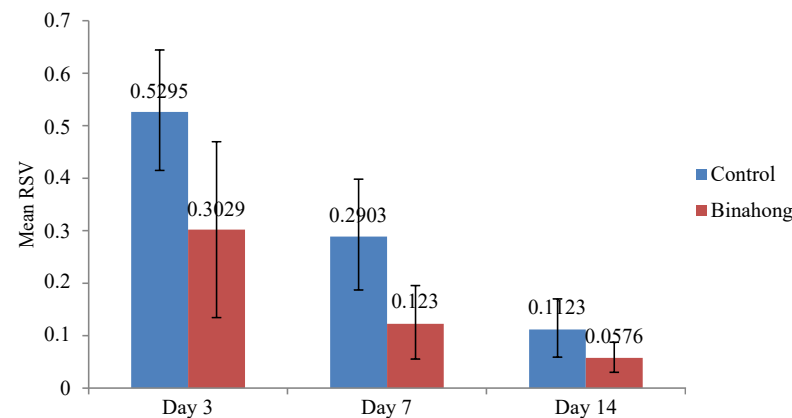


Figure 5. Test results for the difference in the mean RSV between the control and binahong groups.

DISCUSSION

This study was preceded by preliminary *in vivo* and *in vitro* studies by Hanafiah et al.¹³ and Hanafiah et al.¹⁹ regarding binahong leaf extract gel formulation and evaluation on wound healing of the Wistar rat palatal mucosa and its effect on fibroblast proliferation on palatal mucosa wound healing. Based on this study's results, the RSV value, both in the control group and the binahong group, decreased on the 7th and 14th day. This can be caused by the occurrence of fibroplasia which begins during the first week. Fibroplasia is characterised by the growth of fibroblasts and capillaries to migrating epithelium.³ The 7th day of postextraction was the peak of angiogenesis. In the second week postextraction, the socket is filled with granulation tissue and osteoid deposition begins to occur in the alveolar bone.³ The 14th day is the peak of fibroblasts in the wound area.²⁰

The results showed that there was a significant difference in the mean RSV between the control and binahong groups on the 3rd, 7th and 14th days after extraction, while the binahong group showed lower RSV values than the control group. This shows that the wound healing process in the binahong group was better than the control group, which was indicated by the size of the clinical wound shrinking faster. As well as its anti-inflammatory effect, binahong leaf gel can also increase the expression of various growth factors involved in wound healing, increase the proliferation and migration of fibroblasts and have an antibacterial effect.

The study conducted by Hanafiah²¹ regarding the transforming growth factor beta 1 (TGF- β 1) and platelet-derived growth factor two B subunits (PDGF-BB) expression test with immunohistochemistry showed that there was a significant difference between the binahong leaf extract gel group and the control group and the Aloclair® gel group with the control group, where administration of 3% binahong leaf extract gel could increase the expression of PDGF and TGF- β .²¹ Stimulating angiogenesis (mitosis of endothelial cells in functional capillaries), mitogenesis (an increase in cell population for wound healing) and macrophages activation (cleaning the wound area and triggering secondary growth factors for bone regeneration and tissue repair) are the specific functions of PDGF.²² Regulating the proliferation, differentiation, migration, invasion and chemotaxis of epithelial cells, fibroblasts and immune cells (inflammatory phase) of the tissue, as well as the proliferation, migration, invasion and maturation of endothelial cells (to produce functional blood vessels) during angiogenesis are the functions of TGF- β .²³ One of the compounds contained in binahong leaf extract, saponins, can affect the activation and synthesis of TGF- β 1 and is able to modify the TGF- β 1 and TGF- β 2 receptors on fibroblasts. In the remodelling phase, this is an important process for matrix collagen formation. In the proliferative phase, saponins, as angiogenetic agents, can increase the

mitogenic activity of endothelial cells in the formation of blood vessels by regulating VEGF. Based on this, binahong is considered to be able to accelerate wound healing.²⁴

The previous study, conducted by Ardiana et al.,²⁵ showed that giving 5% binahong leaf extract gel was also known to increase the number of fibroblast cells in the socket after tooth extraction. Hanafiah et al.'s¹⁹ *in vitro* study showed binahong leaf extract gel with a concentration of 62.5 ppm can stimulate fibroblast proliferation and shows a greater potential for wound contraction than Aloclair gel with a concentration of 250 ppm in wounds on the palatal mucosa of rats. The results of the scratch wound healing assay *in vitro* also showed that giving binahong leaf extract gel resulted in a better 3T3 fibroblast cell migration acceleration value compared to Aloclair® gel.¹⁹ The increase of fibroblasts stimulates the synthesis of the formation of collagen and extracellular matrix so that new tissue can be formed.^{26,27} In addition, the alkaloid compound from binahong leaf plays a role as an antioxidant and antimicrobial that can help the wound healing process by preventing and protecting the wound area from damage from free radicals and inhibiting the growth of pathogenic bacteria in the wound.²⁸

A study by Leliqia et al.²⁹ showed that binahong leaf extract contains oleanolic acid and ursolic acid. Oleanolic acid and ursolic acid are triterpenoids that are found in many plants. From the results of examination through a scanning electron microscope (SEM), it is known that oleanolic acid can damage bacterial cell membranes.³⁰ Meanwhile, ursolic acid works by destroying the integrity of the bacterial cell membrane at an early stage, which is then followed by inhibition of protein synthesis and various bacterial metabolic pathways.³¹ The limitation of this study was the small sample size because it was conducted during the COVID-19 pandemic era. Further study with a larger number of samples with detailed examination is still needed.

In this study, it was concluded that there was a significant difference in the mean RSV between the control and binahong groups on the 3rd, 7th and 14th day after extraction, where the binahong group showed a lower RSV value than the control group ($p < 0.05$). A 3% binahong leaf extract gel can accelerate the process of post tooth extraction wound healing. Further study is needed regarding the effectiveness of 3% binahong leaf extract gel to post tooth extraction socket healing.

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REFERENCES

- Araújo MG, Silva CO, Misawa M, Sukekava F. Alveolar socket healing: What can we learn? *Periodontol* 2000. 2015; 68(1): 122–34.
- Pagni G, Pellegrini G, Giannobile W V., Rasperini G. Postextraction alveolar ridge preservation: biological basis and treatments. *Int J Dent*. 2012; 2012: 1–13.
- Hupp JR, Ellis E, Tucker MR. Contemporary oral and maxillofacial surgery. 6th ed. St Louis Missouri: Mosby Elsevier; 2014. p. 703.
- Khullar S, A M, Datta P. Healing of tooth extraction socket. *Heal Talk*. 2012; 4(5): 37–9.
- de Sousa Gomes P, Daugela P, Poskevicius L, Mariano L, Fernandes MH. Molecular and cellular aspects of socket healing in the absence and presence of graft materials and autologous platelet concentrates: a focused review. *J oral Maxillofac Res*. 2019; 10(3): e2.
- Farina R, Trombelli L. Wound healing of extraction sockets. *Endod Top*. 2011; 25(1): 16–43.
- Herrera-Briones FJ, Prados Sánchez E, Reyes Botella C, Vallecillo Capilla M. Update on the use of corticosteroids in third molar surgery: systematic review of the literature. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2013; 116(5): e342–51.
- Hernández-Viana S, Silva-Gómez N, Gálvis-Pareja DA, Martínez-Pabón MC. The use of analgesics and anti-inflammatories in an oral surgery service in Medellín, Colombia, 2013-2015. *Rev Fac Odontol*. 2019; 30(2): 154–68.
- Utami P, Puspaningtyas DE. The miracle of herbs. Jakarta: AgroMedia Pustaka; 2013. p. 38–41.
- Hariana A. 262 tumbuhan obat & khasiatnya. Jakarta: Penebar Swadaya; 2013. p. 60–1.
- Mutiara G, Nurdiana, Utami YW. Efektifitas hidrogel binahong (*Anredera cordifolia* (Ten.) Steenis) terhadap penurunan jumlah makrofag pada penyembuhan luka fase proliferasi tikus putih (*Rattus norvegicus*) galur Wistar kondisi hiperglikemia. *Maj Kesehatan FKUB*. 2015; 2(1): 29–40.
- Rathee P, Chaudhary H, Rathee S, Rathee D, Kumar V, Kohli K. Mechanism of action of flavonoids as anti-inflammatory agents: a review. *Inflamm Allergy Drug Targets*. 2009; 8(3): 229–35.
- Hanafiah OA, Trimurni A, Ilyas S, Nainggolan M, Syamsudin E. Formulation and evaluation of binahong leaves extract gel on wound healing of the palatal mucosa. *J Biomimetics, Biomater Biomed Eng*. 2020; 48: 85–91.
- Yüce E, Kömerik N. Potential effects of advanced platelet rich fibrin as a wound-healing accelerator in the management of alveolar osteitis: A randomized clinical trial. *Niger J Clin Pract*. 2019; 22(9): 1189–95.
- Srinivas B, Das P, Rana MM, Qureshi AQ, Vaidya KC, Ahmed Raziuddin SJ. Wound healing and bone regeneration in postextraction sockets with and without platelet-rich fibrin. *Ann Maxillofac Surg*. 2018; 8(1): 28–34.
- Faheem, Samra. Patients compliance and follow-up rate after tooth extraction. *IOSR J Dent Med Sci*. 2017; 16(5): 115–20.
- Motamedi MHK, Navi F, Koushki ES, Rouhipour R, Jafari SM. Hemostatic tampon to reduce bleeding following tooth extraction. *Iran Red Crescent Med J*. 2012; 14(6): 386–8.
- Mozzati M, Gallesio G, di Romana S, Bergamasco L, Pol R. Efficacy of plasma-rich growth factor in the healing of postextraction sockets in patients affected by insulin-dependent diabetes mellitus. *J Oral Maxillofac Surg*. 2014; 72(3): 456–62.
- Hanafiah OA, Abidin T, Ilyas S, Nainggolan M, Syamsudin E. Wound healing activity of binahong (*Anredera cordifolia* (Ten.) Steenis) leaves extract towards NIH-3T3 fibroblast cells. *J Int Dent Med Res*. 2019; 12(3): 854–8.
- Supriatno. Antitumor activity of Papua's *Myrmecodia pendans* in human oral tongue squamous cell carcinoma cell line through induction of cyclin-dependent kinase inhibitor p27Kip1 and suppression of cyclin E. *J Cancer Res Ther*. 2014; 2(3): 48–53.
- Hanafiah OA. Pengaruh pemberian gel ekstrak daun binahong (*Anredera cordifolia* (Ten.) Steenis) dan gel Aloclair® terhadap ekspresi TGFβ1 dan PDGF-BB sebagai penanda proliferasi sel fibroblas pada penyembuhan luka di mukosa palatal tikus Wistar. Dissertation. Medan: Universitas Sumatera Utara; 2019. p. 100–3.
- Shah P, Keppler L, Rutkowski J. A review of platelet derived growth factor playing pivotal role in bone regeneration. *J Oral Implantol*. 2014; 40(3): 330–40.
- Gilbert RWD, Vickaryous MK, Vilorio-Petit AM. Signalling by transforming growth factor beta isoforms in wound healing and tissue regeneration. *J Dev Biol*. 2016; 4(2): 21.
- Gurcharan Singh GK a/p, Utami NV, Usman HA. Effect of topical application of binahong [*Anredera cordifolia* (Ten.) Steenis] leaf paste in wound healing process in mice. *Althea Med J*. 2014; 1(1): 6–11.
- Ardiana T, Kusuma APK, Firdausy MD. Efektivitas pemberian gel binahong (*Adredera Cordifolia*) 5% terhadap jumlah sel fibroblast pada soket pasca pencabutan gigi marmut (*Cavia Cobaya*). *ODONTO Dent J*. 2015; 2: 64–70.
- Kumar V, Abbas AK, Aster JC. Robbins and Cotran pathologic basis of disease. 9th ed. Philadelphia: Elsevier Saunders; 2015. p. 73–91.
- Ågren M. Wound healing biomaterials - volume 1: therapies and regeneration. Duxford: Elsevier; 2016. p. 7–8.
- Agyare C, Dwobeng AS, Agyepong N, Boakye YD, Mensah KB, Ayande PG, Adarkwa-Yiadom M. Antimicrobial, antioxidant, and wound healing properties of *Kigelia africana* (Lam.) Beneth. and *Strophanthus hispidus* DC. *Adv Pharmacol Sci*. 2013; 2013: 692613.
- Leliqia NPE, Sukandar EY, Fidrianny I. Antibacterial activities of *Anredera Cordifolia* (Ten.) V. Steenis leaf extracts and fractions. *Asian J Pharm Clin Res*. 2017; 10(12): 175–8.
- Kim S, Lee H, Lee S, Yoon Y, Choi K-H. Antimicrobial action of oleanolic acid on *Listeria monocytogenes*, *Enterococcus faecium*, and *Enterococcus faecalis*. *PLoS One*. 2015; 10(3): e0118800.
- Wang C-M, Jhan Y-L, Tsai S-J, Chou C-H. The pleiotropic antibacterial mechanisms of ursolic acid against methicillin-resistant staphylococcus aureus (MRSA). *Molecules*. 2016; 21(7): 884.