

The Effect of Topical Application of 3% Binahong (*Anredera cordifolia* (Ten) Steenis) Leaves Extract Gel on the Radiographic Bone Density in Post-Extraction Socket

Olivia Avriyanti Hanafiah^{*1}, Diana Sofia Hanafiah², Rahmi Syaflida¹

1. Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia.

2. Department of Agroecotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia.

Abstract

The use of natural ingredients, either as a medicine or other purpose tends to increase, one of which is the use of *Anredera cordifolia* (Ten) Steenis or binahong plant which is a natural medicinal plant in Indonesia. This study aimed to look at the effect of topical application of 3% binahong leaves extract gel to the relative bone density of post-tooth extraction radiography. Nineteen people were included in this study. After the tooth extraction is completed, the extraction socket was irrigated with sterile saline. Topical application of 3% binahong leaves extract gel performed in the treatment group for 14 days, while no gel application was performed in the control group. Periapical radiograph was taken on the 1st, 7th, 28th, 60th, and 90th days post-extraction. Evaluation of hard tissue healing was done by measuring gray value in digital periapical radiography using ImageJ application. Relative bone density was measured subsequently. The results showed that there were no significant relative density differences between the control and binahong groups on the 1st, 7th and 28th days post-extraction ($p > 0.05$), but there were significant differences on the 60th and 90th days of post-extraction ($p = 0.030$), where the binahong group showed higher bone density than the controls ($p < 0.05$). The increase in relative bone density may be related to the ability of secondary metabolite content in binahong leaves to induce the release of various growth factors that play a role in the process of angiogenesis and bone regeneration. The topical application of 3% binahong leaves extract gel can help the process of hard tissue healing in the post-extraction socket.

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Introduction

Tooth extraction is one of the most common dental and oral treatments in Indonesia.¹ Tooth extraction will induce inflammatory, epithelialization, fibroplasia and remodeling processes, just as occurs in skin or mucosal wounds. However, the post-tooth extraction socket heals by secondary intention, and it can take several months for the socket to fully heal and is difficult to radiographically distinguished from the surrounding bone.²

The use of natural ingredients, either as a medicine or other purpose tends to increase, especially with the concept of "back to nature" as

well as a prolonged crisis that results in a decrease in purchasing ability against modern medicines. This is accompanied by the development of industry in the field of phytopharmacy. Binahong plant is one of the plants that can be used as the raw material in the pharmacy industry.

Anredera cordifolia (Ten) Steenis or binahong plant is a natural medicinal plant in Indonesia. This plant is known as a medicinal plant where parts of binahong plants such as tubers, stems and leaves can be used as herbal therapy. Binahong plants belong to the family Basellaceae.^{3,4} Phytochemical test results of binahong leaves extract showed that binahong leaves contain chemical compounds of saponins, tannins, triterpenoids, alkaloids, flavonoids, phenolics, steroids and glycosides.⁵ Binahong leaves extract also contains vitamin C⁶ and ursolic.⁷ Each of the secondary metabolite content in binahong leaves extract has benefits to the wound healing process. The effect of gel binahong leaves extract on the wound healing

*Corresponding author:

Dr. Olivia Avriyanti Hanafiah,
Department of Oral and Maxillofacial Surgery, Faculty of
Dentistry, Universitas Sumatera Utara, Jl. Alumni. No. 2,
Medan, North Sumatera, Indonesia.
E-mail: olivia.hanafiah@usu.ac.id

process has been quite widely researched, both in animals and humans through clinical, histological, and histochemical examinations, especially on soft tissue healing.⁷⁻¹¹ Binahong can accelerate wound healing through several mechanisms, including by increasing the proliferation and migration of fibroblasts, osteoblasts and expression of various growth factors that are important in wound healing, as well as through anti-bacterial and anti-inflammatory effects.^{5,8-10,12,13} Currently, there is only limited research about the effect of topical application of binahong leaves extract gel on hard tissue healing, especially in post-extraction sockets in human.

The viability test conducted by Hanafiah et al showed that the application of binahong leaves extract does not inhibit the growth of fibroblast cells 3T3 but instead increases its growth. From the study, concentration of 62.5 ppm showed the greatest wound healing potential. The toxic dose of binahong leaves extract against fibroblast 3T3 was 1000 ppm.⁸ Optimization test on binahong leaves extract gel with 3%, 5% and 7% concentration showed that application of 3% binahong leaves extract gel resulted in the highest number of fibroblast cells and greater potential compared to other concentrations⁵. The purpose of this study was to observe the effect of topical application of 3% binahong leaves extract gel on the radiographic relative bone density of post-extraction socket.

Materials and methods

Study Design

This research was conducted in the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Sumatera Utara from June to October 2020. Inclusion criteria in this study were subjects aged 20-30 years, was not taking any medication, did not have any history of systemic diseases that can affect wound healing, indicated for permanent mandibular right or/and left first lower molar extraction, has a good oral hygiene in general. The exclusion criteria in this study were subjects who were not willing to be treated. After screening for the study inclusion/exclusion criteria, a total of 19 participants were included in this study. All participants were randomly selected and divided into control group (n=10) and binahong group (n=9)

Plant Materials and Extract Preparation

Fresh binahong leaves are taken from Simpang Pergendangan village of Tiga Binanga Village Karo Regency, North Sumatra Province. The selected binahong were perfectly opened and were at least 12 weeks old. As much as 400 g of binahong leaves simplisia was placed in a sealed vessel and soaked with ethanol 70% for 5 days at room temperature and protected from light (maceration method). After 5 days, the liquid was removed and filtered with filter paper, then the simplisia was re-soaked with 70% ethanol for 2 days and a final filtration was carried out. Vacuum rotary evaporator and water bath was used to evaporate the solvent until the extract became desiccate. Drugs and reagents such as Carbopol, hydroxypropylmethylcellulose (HPMC), triethanolamine (TEA), glycerin, nipagin (methyl paraben), nipasol (propyl paraben), and distilled water were employed in formulating the Binahong leaves extract gel. All of the materials were of pharmaceutical grade. Nipagin and nipasol were stirred with aquades until homogeneous. This made the first mixture. HPMC were also stirred with aquades until homogeneous and made a second mixture. Both mixtures were stirred together until homogeneous. In another mortar, carbopol, TEA and glycerin were mixed, followed by another stir with the prior mixture. Binahong leaves extract was finally added, mixed and dissolved in sufficient water. Formulation of a 3% gel requires 1.2 grams of extract (3 g/100 g x 40 g).⁵

Tooth Extraction Procedures

Nineteen participants that have met the inclusion criteria were given an explanation of the research procedures. After written informed consent was obtained, all participants received full-mouth scalling to ensure optimal oral hygiene. Prior to the extraction procedure, all vital signs were checked and all participants were instructed to gargle with 0.2% chlorhexidine solution for one minute. Anesthesia was achieved by mandibular blocks and submucous infiltration injections with a 4% articaine (Septanest) of 1:100.000 on the tooth regio to be removed. After clinical signs of anesthesia were seen, operator began the extraction procedures: elevating the attached gingiva around the tooth to be extracted, luxation of tooth from its sockets using elevator, tooth extraction using appropriate forcep, as well as sharp bone smoothing with bone files. Extraction procedures were performed by one operator with

the same procedures.

After the extraction was completed, the extraction socket was irrigated with sterile saline. Topical application of 3 cc 3% binahong leaves extract gel was then performed in the treatment group once a day for 14 days, while no gel application was performed in the control group. Patients were given post-extraction instructions.

Radiographic Relative Bone Density Measurement

Periapical radiography was taken on the 1st, 7th, 28th, 60th, and 90th days post-extraction for evaluation of hard tissue healing. Periapical radiography was taken using portable X-Ray tools (Rextar X, Posdion Co., Ltd, Korea) and digitalized using software and digital films (EzDent-I, Vatech, Korea). Evaluation of hard tissue healing was done by measuring bone density in digital periapical radiography using imageJ application (U. S. National Institutes of Health, Maryland, USA). The mean bone density value was obtained by measuring the mean gray value in the socket area after tooth extraction in each follow-up period as described by Geiger et al.¹⁴ Gray value measurement steps in ImageJ application are as follows:

a) Open the app. Set measurement parameters on analyze drop down menu → set measurements → check area and Mean gray value.

b) Open the digitized radiographic image by clicking Open File → file location

c) Determine the boundaries of the socket area to be measured (region of interest /ROI) with the free selection tools feature. Outline of the same socket was copied to the subsequent radiograph taken on days 7,28,60 and 90 post-extraction so that measurements were actually done on the same area.

d) After the ROI determination was completed, click Analyze → Measure to get the mean gray value.

e) Determine the boundaries of the bone area around the socket to be used as control (region of control /ROC) with the free selection tools feature, then click Analyze → Measure to get the mean value of gray of the control area.

f) Relative density is calculated by the formula:

$$\frac{\text{mean gray value on ROI}}{\text{mean gray value on ROC}}$$

Radiographic evaluation results were recorded. The repeated ANOVA and Friedman

test were used to compare relative bone density mean in each group, while independent samples t-test and Mann-Whitney test were used to compare relative bone density mean between the groups at each interval. All statistical analysis was performed with the statistical package for the social sciences (SPSS), version 21 (IBM® Inc, New York, USA). The difference in data was considered significant if the p-value was less than 0.05.

The research protocol was approved by the Health Research Ethical Committee, Universitas Sumatera Utara, Medan, Indonesia (55/KEP/USU/2020).

Results

Table 1 shows that in the control group, the mean relative bone density of alveolar bones in the socket increased at each measurement interval. Repeated ANOVA test results showed that there was a significant change in the overall relative bone density of post extraction socket in control group. Subsequent paired wise comparison test results showed significant differences in each measurement (p<0.05).

| Day | Mean relative bone density ±SD | p-value |
|-----|--------------------------------|---------|
| 1 | 0,6832±0,0827 | 0,002 |
| 7 | 0,7089±0,0804 | |
| 28 | 0,7294±0,0753 | |
| 60 | 0,7479±0,0743 | |
| 90 | 0,7688±0,0718 | |

Table 1. Repeated ANOVA test results for relative bone density of post-extraction sockets in control group.

Tabel 2 shows that in the binahong group, relative bone density values also increased at each measurement interval. Friedman test results also showed there was a significant change in the relative bone density of the post-extraction socket (p= 0.001) in binahong group. Post hoc test results with Wilcoxon test and dependent samples t-test showed significant changes in relative bone density at each measurement (p<0.05).

Tabel 3 shows mean comparison of relative bone density between control group and binahong group. Independent samples t-test results showed no significant difference in relative bone density between control group and binahong group on the 1st, 7th and 28th days

post-extraction ($p>0.05$), but there was a significant difference on the 60th day of post-extraction ($p=0.030$). Mann-Whitney test results also showed significant differences in the relative bone density between the control group and binahong on the 90th day after extraction ($p=0.003$).

| Day | Mean relative density \pm SD | <i>p</i> -value |
|-----|--------------------------------|-----------------|
| 1 | 0,7430 \pm 0,0715 | 0,001 |
| 7 | 0,7665 \pm 0,0785 | |
| 28 | 0,7891 \pm 0,0684 | |
| 60 | 0,8273 \pm 0,0716 | |
| 90 | 0,8779 \pm 0,0637 | |

Table 2. Friedman test results for relative bone density of post-extraction sockets in binahong group.

| Day | Mean relative density \pm SD | | <i>p</i> -value |
|-----|--------------------------------|---------------------|-----------------|
| | Control | Binahong | |
| 1 | 0,6832 \pm 0,0827 | 0,7430 \pm 0,0715 | 0,11 |
| 7 | 0,7089 \pm 0,0804 | 0,7665 \pm 0,0785 | 0,13 |
| 28 | 0,7294 \pm 0,0753 | 0,7891 \pm 0,0684 | 0,09 |
| 60 | 0,7479 \pm 0,0743 | 0,8273 \pm 0,0716 | 0,03 |
| 90 | 0,7688 \pm 0,0718 | 0,8779 \pm 0,0637 | 0,003 |

Table 3. Mean comparison of relative bone density of post-extraction socket between control group and binahong group. Preoperative appearance.

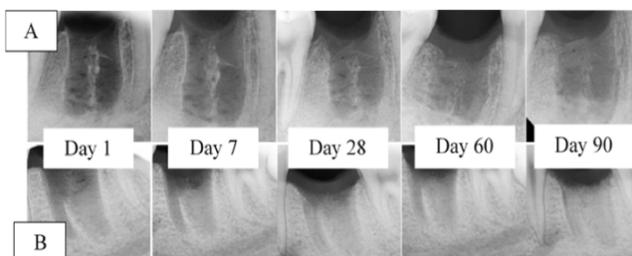


Figure 1. Radiographic changes of post-extraction sockets on (A) control group and (B) binahong group. Statistical test results showed that there was a significant difference in the mean relative bone density at day 60 and 90 post extraction.

Discussion

Based on previous study, binahong is known to contain quercetin, a flavonoid that is proven to increase ALP and accelerate bone mineralization.^{10,15} Study by Wong et al showed that quercetin has biological potential for new bone formation in animal bone defects within 14 days.^{15,16} Therefore, we decided to do the application of binahong leaves extract gel for 14

days.

The statistical results showed that there was no significant differences in the relative bone density between control group and binahong group on day 1, 7th and 28th post-extraction ($p>0.05$). It can be concluded that the initial (baseline) bone density was the same for both groups. Meanwhile, in the 60th and 90th days post-extraction, binahong group showed a significantly higher relative bone density than control ($p<0.05$). This study is the first study to show the effectiveness of 3% binahong leaves extract gel application in increasing radiographic bone density of post tooth extraction socket.

The process of healing the alveolar bone after tooth extraction involves osteoblast activity.¹⁷ In addition to its ability to increase the number of fibroblasts, the application of binahong leaves extract gel is also known to increase the number of osteoblasts in post-extraction sockets. Studies done by Sa'diyah et al and Khoswanto showed that the application of 5% and 10% binahong leaves extract gel can increase the number of osteoblasts in post-extraction sockets.^{10,11} This indicates that binahong leaves extract gel is able to accelerate the proliferation and differentiation of osteoprogenitor cells into osteoblast cells.¹¹ This might be due to increased expression of bone morphogenetic protein-2 (BMP-2) which occurs after the administration of binahong leaves extract gel on post-extraction sockets.¹⁰ BMP may induce the expression of osteoblast markers and stimulate bone formation. BMP also plays an important role in the differentiation, proliferation and morphogenic processes of bone and cartilage.¹⁸ Binahong also contains vitamin C, which may play a role in the healing process of alveolar bone. Study done by Indrati and Shinta showed that vitamin C has the ability to stimulate osteoblast differentiation through alkaline phosphatase (ALP) stimulation.¹⁷ ALP is a cell membrane enzyme produced by osteoblasts. The production of ALP bone increases in the process of bone formation so that it can be an excellent bone formation marker.¹⁹ Vitamin C also increases the expression of BMP-2, osteocalcin, collagen type I, as well as prevent osteoclastogenesis.²⁰

As previously mentioned, quercetin, a flavonoid presented in binahong, was proven to increase ALP and accelerate bone mineralization.^{15,16} In addition, quercetin is also known to induce angiogenesis processes.²¹

Angiogenesis is one of the important processes that occur during the healing process of tooth extraction sockets. Through this process, the inflammatory cells, growth factors, and progenitor cells needed during the inflammatory and proliferative process will be fulfilled. In addition, alveolar bone regeneration and healing also depends on the process of angiogenesis.²² In the process of bone repair, angiogenesis occurs first compared to osteogenesis, because bone regeneration itself can not successfully occurred in the absence of blood supply to the defect area, so angiogenesis process is absolutely important for bone regeneration. Study by Zhou et al showed that quercetin can increase cell proliferation, osteogenic differentiation and secretion of angiogenic factors from rat bone marrow-derived mesenchymal stem cells, one of which is vascular endothelial growth factor (VEGF). As a key factor in the angiogenic process, VEGF can induce osteogenesis and angiogenesis simultaneously. VEGF has a specific role to vascular endothelial cells by inducing cell proliferation and migration, as well as inducing angiogenesis.²¹

Meanwhile, research done by Khoswanto showed that the application of binahong gel can increase the expression of hypoxia-inducible factor 1 α (HIF-1 α). HIF-1 α can induce angiogenesis by inducing vascular endothelial growth factor (VEGF-A) expression as a direct target of HIF-1 α . In addition, HIF-1 α also increased the expression of BMP-2. This increase in HIF-1 α expression is also associated with quercetin compound contained in binahong plants.^{9,23}

Conclusions

There was a significant difference in relative bone density on the 60th and 90th days of post-extraction ($p=0.030$) between control and binahong group, where binahong group showed a higher bone density than the control group ($p<0.05$). It can be concluded that the topical application of 3% binahong leaves extract gel can help the process of hard tissue healing in the post-extraction socket. Further research is needed to produce better and stable gel formulations and clinical trials are carried out on larger samples.

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Declaration of Interest

The authors report no conflict of interest.

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