

RESEARCH ARTICLES

Pengaruh Pemberian Gel Ekstrak Daun Binahong (*Anredera cordifolia*) 5% terhadap Aktivitas Makrofag dalam Penyembuhan Luka Pasca Pencabutan Gigi pada Tikus Wistar

Effect of 5% Binahong (*Anredera cordifolia*) Leaf Extract Gel on Macrophage Activity in Wound Healing After Tooth Extraction in Wistar Rats
Naila Fadhilah Romadhona^{1*}, Nilasary Rochmanita Suparno², Nendika Dyah Ayu Murika Sari³, Juwita Raditya Ningsih⁴

^{1,2,3,4} Dentistry Study Program, Faculty of Dentistry, University of Muhammadiyah Surakarta, Surakarta, Indonesia

Abstract

Tooth extraction is an action that is often performed in the world of dentistry to remove intact teeth or remaining roots from the sockets in the alveolar bone. Macrophage cells are important processes in wound healing during the inflammatory phase. Binahong leaves (*Anredera cordifolia*) contain flavonoids, ascorbic acid, tannins, terpenoids, saponins, alkaloids which influence macrophage activity. The aim of the research was to determine the effect of 5% binahong (*Anredera cordifolia*) leaf extract gel on the increase in macrophages after tooth extraction in Wistar strain white rats. This type of research was pure experimental research with a post test only group design. The research subjects were 36 mice divided into negative control, positive control and treatment groups. The left mandibular incisor was removed and CMC-NA 1%, glycerin iodine 1%, and 5% binahong (*Anredera cordifolia*) leaf extract gel were applied. The lower incisors of mice were extracted and then made histological preparations stained with hematoxylin then divided into 3 groups, then the number of macrophages was counted using a microscope with 400x magnification. The One Way Anova parametric test showed that there was a significant difference ($p < 0,05$) between groups in the number of macrophages. The results of the LSD test showed that there was a significant difference ($p < 0,05$) between the treatment group and the positive and negative controls. Based on this research, 5% binahong leaf extract gel has an effect on the number of macrophages cells in the wound healing process after tooth extraction.

Keywords: binahong extract, herbal medicine, macrophages, tooth extraction

Article history:

Submitted 23 March 2024

Accepted 30 December 2024

Published 31 December 2024

PUBLISHED BY:

Sarana Ilmu Indonesia (salnesia)

Address:

Jl. Dr. Ratulangi No. 75A, Baju Bodoa, Maros Baru,
Kab. Maros, Provinsi Sulawesi Selatan, Indonesia

Email:

info@salnesia.id, jika@salnesia.id

Phone:

+62 85255155883



Abstrak

Pencabutan gigi merupakan tindakan yang sering dilakukan di dalam dunia kedokteran gigi untuk mengeluarkan gigi utuh atau sisa akar dari soket pada tulang alveolar. Sel makrofag merupakan proses penting pada penyembuhan luka pada fase inflamasi. Daun binahong (*Anredera cordifolia*) memiliki kandungan berupa flavonoid, asam askorbat, tanin, terpenoid, saponin, alkaloid yang mempengaruhi aktivitas makrofag. Tujuan penelitian adalah untuk mengetahui pengaruh gel ekstrak daun binahong (*Anredera cordifolia*) 5% terhadap peningkatan makrofag pasca pencabutan gigi tikus putih Strain Wistar. Jenis penelitian ini merupakan penelitian eksperimental murni dengan rancangan post test only group design. Subjek penelitian 36 ekor tikus dibagi menjadi kelompok kontrol negatif, kontrol positif, dan perlakuan. Pencabutan gigi insisivus kiri rahang bawah dan dilakukan pengaplikasian CMC-Na 1%, iod gliserin 1%, dan gel ekstrak daun binahong (*Anredera cordifolia*) 5%. Gigi insisivus bawah tikus diekstraksi dan dibuat sediaan histologis yang diwarnai dengan hematoksilin lalu dibagi menjadi 3 kelompok, kemudian dihitung jumlah makrofag menggunakan mikroskop dengan perbesaran 400x. Uji parametrik One Way Anova menunjukkan terdapat perbedaan yang bermakna ($p < 0,05$) antar kelompok pada jumlah makrofag. Hasil uji LSD menunjukkan terdapat perbedaan yang bermakna ($p < 0,05$) antara kelompok perlakuan dengan kontrol positif dan negatif. Berdasarkan penelitian tersebut gel ekstrak daun binahong 5% berpengaruh terhadap jumlah sel makrofag pada proses penyembuhan luka pasca pencabutan gigi.

Kata Kunci: ekstrak binahong, obat herbal, makrofag, pencabutan gigi

*Correspondence Author:

Naila Fadhilah Romadhona, email: nailaromadhona3@gmail.com



This is an open access article under the **CC-BY** license

INTRODUCTION

Tooth extraction is an action that is often done in the world of dentistry to remove intact teeth or residual roots from sockets in the alveolar bone. This is the last alternative if the condition of the teeth cannot be maintained with other types of treatment (Kurniawati *et al.*, 2024). Meanwhile, wounds are damage to body tissues or tissue components that contain damaged or missing tissue substance. Slow healing of depilation wounds can cause sufferers to experience complaints such as pain, swelling, bleeding, impaired masticatory function, impaired speech function, and even infection (Kresnoadi, 2013).

The wound healing process is a process that involves biochemical and physiological phenomena and requires complex biological sequences. The wound healing process has four phases, namely the hemostatic phase, the inflammatory phase, the proliferation phase, and the remodeling phase. The inflammatory phase is a series of hemostasis reactions followed by inflammatory reactions which include vasodilation and increased capillary permeability. In the inflammatory phase, a vasoconstriction process occurs, shrinking the ends of broken blood vessels (retraction) (Arief *et al.*, 2016). The inflammatory phase begins when a wound occurs and lasts for 1-3 days. During inflammation, wounds show signs of inflammation such as redness, swelling, heat and pain (Arief *et al.*, 2016). The mechanism of the inflammatory phase in wound healing, formed by free radicals Reactive Oxygen Species (ROS) produced by neutrophils and macrophages as part of the immune system, helps accelerate wound cleaning from bacterial attacks (Arief *et al.*, 2016). The inflammatory phase in wound healing is macrophage cells that appear 24-48 hours post-trauma and reach their peak on

day 7 (Prameswari *et al.*, 2022).

Macrophage cells play a role in maintaining normal tissue by phagocytosis. In addition, secreting cytokines and other trace objects then breaking down these cells with lysosomal enzymes (Primidina *et al.*, 2019). To accelerate the inflammatory phase can be given NSAID anti-inflammatory drugs and glycerin iodine. The advantage of using this anti-inflammatory drug can be used because its dosage form is oral, can relieve inflammatory symptoms, easily available in the market because it is sold freely like mother profen (Ghlichloo dan Gerriets, 2023).

The use of anti-inflammatory drugs in a long time will result in dangerous side effects such as causing disorders of the digestive tract such as stomach, ulcers, and impaired kidney function (Latief *et al.*, 2021), besides that glycerin iodine has side effects in some patients, such as irritation, sensitivity reactions, iododerma, pain, and inhibiting fibroblast determination in long-term use. Therefore, alternative medicine is needed to accelerate the inflammatory phase in the wound healing process after tooth extraction using traditional medicine whose basic ingredients are herbal plants. Traditional medicine is widely used by the public because it is easy to find, the price is more economical, and has minimal side effects compared to chemical drugs.

One of the herbal plants that have been used to treat wounds is the binahong plant (*Anredera cordifolia*) originating from South America which empirically has benefits for analgesics, wound healing, and therapy of various diseases such as diabetes mellitus. Almost all parts of binahong plants can be utilized, starting from stems, roots, flowers, and leaves, but the part that is widely used as herbal medicinal materials is the leaves.

According to (Mokondongan *et al.*, 2017) the chemical content contained in binahong plants (*Anredera cordifolia*) consists of flavonoids, ascorbic acid, tannins, terpenoids, saponins, alkaloids. The active compounds of flavonoids can help differentiate monocytes into macrophages and then phagocytes when there are antigens entering. Ascorbic acid is anti-inflammatory which can strengthen the resistance of cells to infection so that they can regenerate properly. Tannins can prevent damage to the wound area due to free radicals and prevent the growth of pathogenic bacteria on the wound. Terpenoids as antioxidants that will inhibit the work of macrophage cells. Saponins can fight antigens so that they can ease the work of inflammatory cells such as macrophage cells when the body has antigens. Alkaloids have anti-inflammatory properties that can strengthen cell resistance to infection so that they can accelerate the wound healing process (Hanafiah *et al.*, 2021).

Among the community, binahong leaves are used to treat pain, ulcers, canker sores, give extra stamina, improve blood circulation, and gout. In addition, consuming binahong leaves can also overcome swelling and blood clots, lower cholesterol, and heal wounds. Binahong leaves can be made in the form of gel preparations because the gel is non-sticky, non-greasy, gives a cold feeling on the skin because there is a high enough water content so that it is comfortable to use on the skin, besides that the gel is easy to apply to the socket of white wistar rats (*Rattus novergicus*) (Anggraini *et al.*, 2016).

Previous research by Sa'diyah *et al.* (2019) on the application of binahong leaf extract gel concentration of 5% on wounds after tooth extraction of wistar white rats (*Rattus novergicus*) found that 5% binahong leaf extract gel had an effect on osteoblast wounds after tooth extraction of wistar white rats (*Rattus novergicus*). The result was an influence on the increase in osteoblasts in rats given 5% binahong leaf extract gel. Osteoblasts are formed from the proliferation of osteoprogenitor cells in the periosteum and bone marrow (Koraag *et al.*, 2015), therefore, researchers feel the need to know the effect of 5% binahong leaf extract gel on the number of macrophages in the wound

healing process because macrophages are one of the inflammatory cells that play a role in the wound healing process.

Binahong plant



Fig 1. Binahong plant (*anredera cordifolia*) (Kotala dan Kurnia, 2022).

Binahong plant content (*Anredera cordifolia*)

Flavonoid: Flavonoids work to increase the production of the cytokine IL-2. The IL-2 cytokine can affect CD4+ and then cause Th1 cells to be activated. Activated Th1 cells will affect IFN- γ (Interferon γ). This IFN- γ cytokine will activate monocytes to become macrophages, and has the ability to increase the number of macrophages and then phagocytose when antigens enter the body (Kotala dan Kurnia, 2022).

Ascorbic acid: Ascorbic acid has anti-inflammatory properties which can strengthen cells resistance to infection so that cells can regenerate well (Susetya *et al.*, 2014). *Tannins*: Tannins act as antioxidants and antimicrobials which can help the wound healing process by preventing and protecting the wound area from free radical damage (Yuniarti dan Lukiswanto, 2017).

Terpenoid: Terpenoids in binahong leaves are used as antioxidants to inhibit the work of macrophage cells. *Saponins*: Saponin works to fight antigens so that it can relieve the work of inflammatory cells such as macrophage cells, neutrophil cells and lymphocyte cells when antigens are present in the body (Sharma *et al.*, 2020).

Alkaloid: Ascorbic acid is an important factor in the formation of the prolyl hydroxylase enzyme which functions for collagen biosynthesis in the wound healing process (Ravetti *et al.*, 2019).

Gel

Gel is a topical preparation that uses a polar base formulation so that it is easily accepted by the skin and the diffusion power caused is better than cream because of its ability to pass through the skin membrane more effectively than cream preparations. Gelling agent is the basic material for making gels that have various types including carbopot, carboxymethyl cellulose (CMC) and (HPMC) (Ani *et al.*, 2018). Gelling components can absorb solutions so that volume increases, very high electrolyte concentrations can affect hydrophilic gels where ions compete effectively with colloids against existing solvents and colloids are salted (dissolved), gel structure forms resistant to change or deformation and has viscoelastic flow. Has high viscosity and adhesion so that it does not flow easily on the surface of the skin, has thylotropic properties so that it is easily evenly distributed when applied, does not leave marks, only in the form of a thin layer like a film when using, easily washed with water, and gives a cold sensation

after use, able to penetrate further than cream (Anggraini *et al.*, 2016). Very easy to wash and easy to lose when the skin sweats, the price is more expensive, must use active substances that dissolve in water so that it is necessary to use increased solubility such as surfactants to remain clear at various temperature changes.

Tooth extraction

Tooth extraction or exodontia is a science that studies good, hygienic and painless tooth extraction accompanied by good complication management before, during and after the action (Sitanaya, 2016). The ideal tooth extraction is the extraction of one intact tooth or tooth root with trauma to the supporting tissue of the tooth so that the extraction scar can heal completely. This action results in damage to soft and hard tissues. After tooth extraction, the alveolar socket will be filled with blood clots which will then be replaced by granulation tissue for 7 days.

Post-tooth extraction wounds

Wound is a form of tissue damage to the skin caused by contact with heat sources (such as chemicals, hot water, fire, radiation, and electricity), the results of medical actions, or changes in physiological conditions. Wounds cause disturbances in the functions and anatomical structures of the body. Based on the timing and healing process, wounds can be classified into acute and chronic wounds. Acute wounds are tissue injuries that can recover to normal conditions with minimal scarring in a span of 8-12 weeks. While chronic wounds are wounds with a slow recovery process, with a healing time of more than 12 weeks and can sometimes cause disability (Purnama *et al.*, 2017).

Macrophage

Macrophages are one of the cells that play a role in the wound healing process. On the third day of the wound, monocytes differentiate into macrophages into the wound through the mediation of monocyte chemoattractant protein 1 (MCP-1). Macrophages are \pm 9-12 μ m in size and only a few in number. Macrophages as cells that are very important in wound healing have the function of phagocytosis of bacteria and dead tissue will turn into macrophage efferocytosis (M2) which secretes anti-inflammatory cytokines such as IL-4, IL-10, IL13 (Landén *et al.*, 2016). Macrophages secrete proteinases to degrade the extracellular matrix (ECM) and are important for removing foreign material, stimulating cell movement, and regulating ECM turnover.

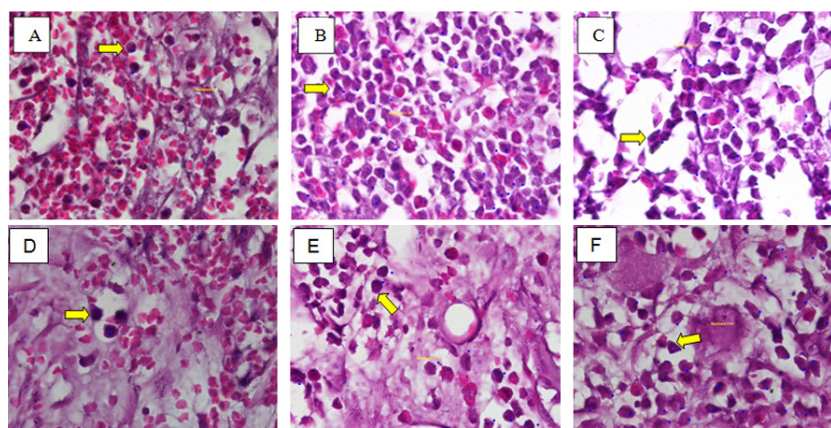


Fig 2. Macrophages (Budi *et al.*, 2017).

White rat (*Rattus Novergicus*) wistar strain

Taxonomy of white rats (*Rattus Novergicus*) wistar strains

White rats are animals originating from China which then spread in western Europe. And in Southeast Asia, white rats breed in the Philippines, Indonesia, Laos Singapore and Malaysia (Angria, 2019). The taxonomy that has been researched by (Nugrahwati, 2016) is as follows:

Kingdom : Animalia
Filum : Chordata
Class : Mammals
Ordo : Rodentia
Edge : Odontoceti
Familia : Muridae
Genus : Rattus
Species : *Rattus norvegicus*
Strain : *Wistar*



Fig 3. White rat (*Rattus norvegicus*) Wistar strain (Al-Hajj *et al.*, 2016).

Theoretical foundation

One of the dentistry procedures is tooth extraction treatment with the aim of removing teeth from the alveolar bone. Tooth extraction causes injury to the tooth socket and blood will flow around the tooth due to the rupture of blood vessels and will experience a wound healing process. The wound healing process, especially in the oral mucosa, there are four phases that must be passed, namely the hemostatic phase or blood clotting, the inflammatory phase, the proliferation phase, and the remodeling phase. The four phases are overlapping phases.

This hemostatic phase will stop bleeding through vasoconstriction and blood clotting to close blood vessels. In the inflammatory phase, neutrophil cells have an important role to eliminate infection and are drawn into the wound area by components of the complement system. Neutrophils will work together with macrophages together to remove bacteria, dead cells, apoptotic neutrophils, tissue residue and other foreign bodies. Neutrophils are then replaced by macrophages at 48-72 hours after injury to continue the phagocytosis process.

The inflammatory phase involves macrophage cells because they have the function of phagocytosis, releasing growth factors and cytokines which then attract cells that play a role in the proliferation phase to the wound site and stimulate cells that play a role in the process of wound reepithelialization, create granulation tissue, and make up the extracellular matrix. The purpose of the proliferation phase is the balance between scar tissue or scar formed with tissue regeneration in the wound area. Then enter the last phase, namely the remodeling phase which lasts for 14 to 1 year or 2 years or even more. The remodeling phase aims to increase the strength of new epithelial tissue and

the scar tissue formed.

There are several drug options that can be consumed after tooth extraction to accelerate the inflammatory phase, such as steroid and non-steroidal anti-inflammatory, and glycerin iodine. However, these drugs have contraindications that can cause irritation and pain when used for a long time. Therefore, herbal medicine is needed as an alternative to accelerate the healing process of wounds after tooth extraction.

Binahong plant (*Anredera cordifolia*) is known as an herbal plant that is widely found in Indonesia. This plant contains binahong leaves such as flavonoids, saponins, tannins, terpenoids, and ascorbic acid which serves to accelerate the inflammatory phase. All ingredients in binahong leaves have the potential to heal wounds after tooth extraction and are able to stimulate the wound area so that macrophage inflammatory cells accelerate the wound healing process. The concentration of binahong leaf extract gel preparation (*Anredera cordifolia*) 5% has been proven effective for the wound healing process. A concentration of 5% is expected to affect the number of macrophages in the wound healing process after tooth extraction of white rats (*Rattus norvegicus*) Wistar strain so that it can accelerate the wound healing process.

METHODS

The type of research carried out is pure laboratory experimental research with a post test only control group design. Variable independent is Binahong leaf extract gel (*Anredera cordifolia*) concentration 5, variable dependencies is the number of macrophage cells on days 3, 5, 7 and 14 in the wound healing process after tooth extraction of white rats (*Rattus norvegicus*) Wistar strain in histological preparations with hematoxylin eosin staining and Controlled variables: Magnification of the microscope used, Field of view observed, Macrophage cell tagging. The study will be carried out by observing and calculating the number of macrophage cells in HPA preparations carried out at the Anatomical Pathology Laboratory of the Faculty of Medicine, Sebelas Maret University in January 2024.

In this study the research, subjects to be used were 36 jaw preparations of white rats (*Rattus norvegicus*) male Wistar strains which were divided into 3 treatment groups, the first group was a negative control group with CMC-Na 1%, the second group was a positive control group of 1% glycerin iodine and the third group was a 5% binahong extra gel treatment group, Each group consisted of Each group consisted of 9 jawbone preparation of white rat (*Rattus norvegicus*) Wistar strain from days 3, 5, 7, and 14. Code A was used as labeling of the negative control group preparation, code B for the positive control group, and group C for the 5% binahong extract gel treatment group. While the labeling for days is given the code H3 for day 3, H5 for day 5, H7 for day 7, and H-14 for day 14.

The object of this study was the number of macrophages in histological preparations on days 3, 5, 7, and 14 after tooth extraction of white rats (*Rattus norvegicus*) Wistar strains. Calculation of the number of macrophages through histological preparations viewed using a Leica ICC50E microscope. The resulting image with a magnification of 400x as much as 5 fields of view is then connected to leica application image software, then the calculation of the number of macrophages will be carried out,

RESULTS AND DISCUSSIONS

The results showed that the calculation of the average and standard deviation in

each group was presented in Table 1 and Figure 4.

Table 1. Average and standard deviation of the number of macrophage cells

Observation day	Average ± Standard Deviation		
	Negative Control	Positive Control	Treatment
3	85,33±28,75	87,33±42,00	60,67±50,29
5	69,67±34,196	93.00±29.05	86,00±45,92
7	101.00±35.03	175.67±26.10	121,67±54,86
14	56,33±61,84	54,33±8,08	66,33±37,018

Based on the graph below, it can be seen that there is a change in the average value and standard deviation of each group in each observation.

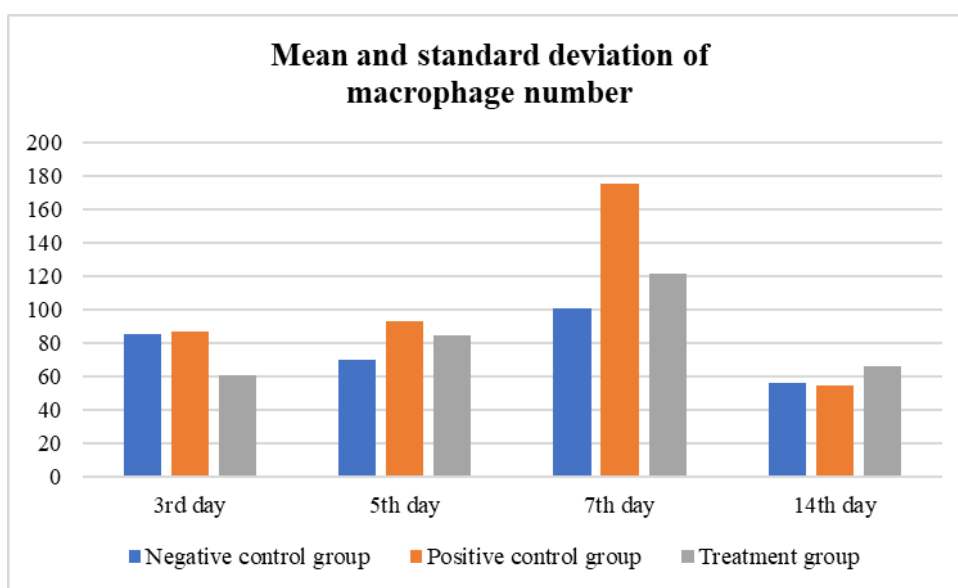


Fig 4. Graph of the average and standard deviation of the number of macrophage cells

The data above showed that the positive control group on day 7 had the highest average number of macrophages with an average of 175,67, then decreased on day 14 with an average of 54,33. The negative control group on day 7, the highest average macrophage count was 101,00, then decreased on day 14 with an average of 56,33.

The average number of macrophages in the treatment group on day 3 with an average of 60,67, on day 5 with an average of 86,00, on day 7 with an average of 121,67, it can be said that on day 3 to day 7 has increased. Furthermore, the number of macrophages decreased on day 14 with an average of 66,33. The graph of the average number of macrophage cells in the 5% binahong extract gel treatment group only had 1 peak on day 7 indicating that the number of macrophage cells only rose on day 7 after tooth extraction after which it decreased. The difference in the number of macrophage cells is the influence of the active compound in binahong leaf gel extract 5% which affects macrophage cells in the wound healing process after tooth extraction of Wistar strain rats.

The standard deviation value depicted by the error bar shows that in the positive

control group is the largest value obtained when observing day 7, in the negative control group experienced an increase in value on day 3 then decreased on day 5, then increased again on day 7 and on day 14 decreased again.

The macrophage image obtained is purplish blue, the nucleus is larger than neutrophil cells and lymphocytes, and shaped like coffee beans as Figure 5.

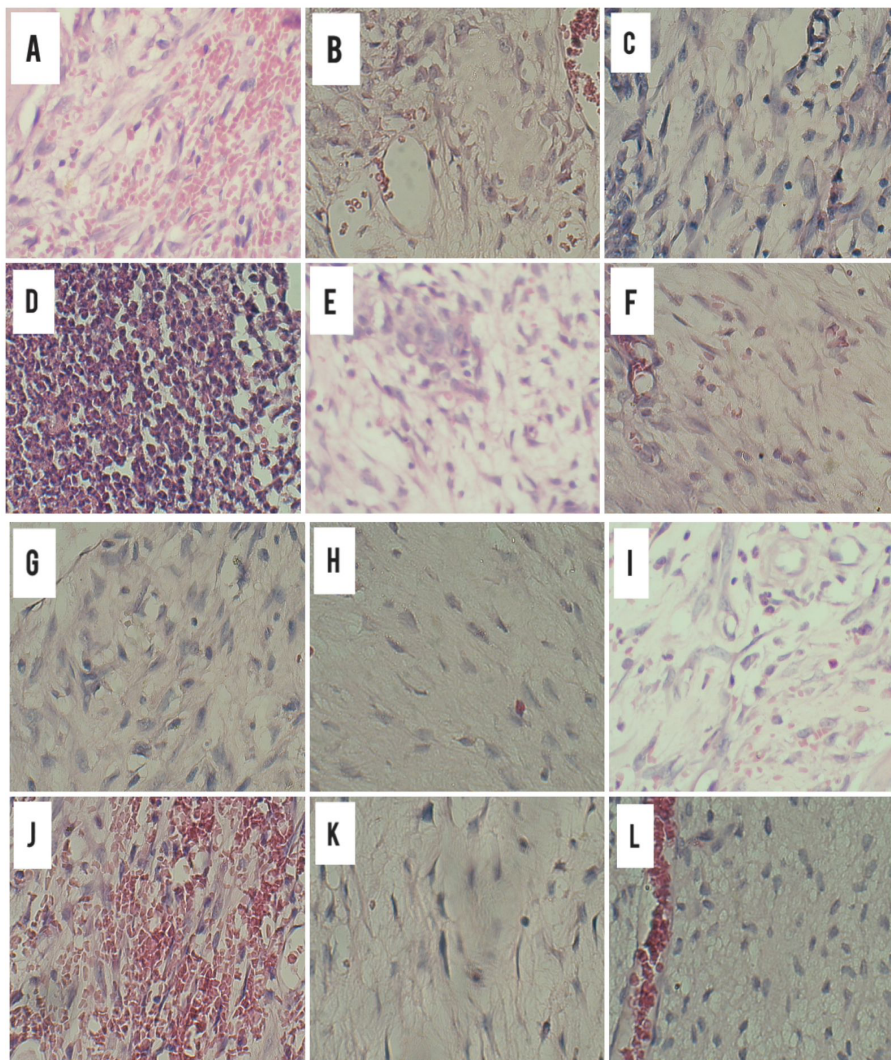


Fig 5. (A) 3rd day negative control, (B) 3rd day positive control, (C) 3rd day treatment; (D) 5th day negative control, (E) 5th day positive control, (F) 5th day treatment; (G) 7th day negative control, (H) 7th day positive control, (I) 7th day treatment; (J) Negative control day 14, (K) Positive control day 14, (L) Treatment day 14; (400x magnification).

Based on the normality test results from the data measured through SPSS 22 software as a whole, it can be seen that the Table 1 shows the results of the Normality Test data regarding the average preparation on each observation day using the Shapiro-Wilk normality test. This test was chosen based on the number of samples in this study, namely with a total of <50 samples or as many as 36 samples.

Table 2. Normality test data on the number of macrophage cells of rat jaw preparations

Data	Shapiro-Wilk		
	Statistic	df	Sig.
Average 3rd day preparation	0,842	9	0,061
Average 5th day preparation	0,886	9	0,181
Average 7th day preparation	0,940	9	0,578
Average 14th day preparation	0,949	9	0,648

Based on these results, the normality test results can be seen through the p-value (Sig). The p-values produced on observations on days 3, 5, 7, and 14 showed a number of >0,05 (p-value = 0,061; 0,181; 0,578; and 0,648), which means that all observation data have been normally distributed and meet the normality test. The normally distributed data was then followed by a homogeneity test using Levene 's Test. The homogeneity test serves to determine the variation between groups of the same or variations from homogeneous data.

Table 3. Test the homogeneity of macrophage cell count data.

Data	Levene Statistic	df1	df2	Sig.
Average 3rd day preparation	0,951	2	6	0,438
Average 5th day preparation	0,866	2	6	0,467
Average 7th day preparation	1,566	2	6	0,284
Average 14th day preparation	3,163	2	6	0,115

The Table 3 shows the results of the homogeneity test of the average data variance of the preparation on each day of observation. The homogeneity test results can be seen based on the p-value (Sig) based on mean. In the table, the p-values generated on days 3, 5, 7, and 14 of observation, respectively show a value of > 0,05 (p-value = 0,438; 0,467; 0,284; and 0,115), which means that all data variances on each day of observation are homogeneous.

Furthermore, parametric tests were carried out using the One Way Anova test. Parametric tests using One Way Anova aim to find out whether each group has meaningful differences where there is at least one group that has meaningful differences. Parametric test using One Way Anova if >0,05 is said to have no difference, and if <0,05 it is said to be a difference.

Table 4. Parametric test results One Way Anova

Day	p-value
3	0,029*
5	0,008*
7	0,042*
14	0,029*

Description: * One Way Anova test, (p-value <0,05) : there is a meaningful difference

Based on the results of the One Way Anova parametric test on days 3, 5, 7, 14 showed a p-value of <0,05 so it can be said that the data results have significant differences between groups. This shows that binahong extract gel (*Anredera cordifolia*) 5% has an influence on the number of macrophage cells in the wound healing process after tooth extraction of white rats (*Rattus novergicus*) Wistar strains. The next test is

the Post Hoc test using LSD (Least Significant of Difference) to determine meaningful differences between groups

Table 5. LSD analysis of intergroup macrophage cell counts

Group	Observation Day	p-value
Treatment-Negative Control	3	0,065
	5	0,099
	7	0,513
	14	0,048*
Treatment-Positive Control	3	0,011*
	5	0,003*
	7	0,046*
	14	0,011*

Description: * One Way Anova test, (p-value <0,05) : there is a meaningful difference

The results of the Post hoc LSD test above showed that on days 3, 5, 7, and 14 there was a significant difference between the positive control group and the treatment group by showing a p-value of <0,05. This showed that there was an effect of giving binahong extract gel 5% on the number of macrophage cells in the wound healing process after tooth extraction of white rats Wistar strain compared to a positive control group using glycerin iodine.

From the Table 5, it can be seen that on the 3rd, 5th, 7th day the p-value >0,05 which means that there is no difference between the treatment group and the negative control group. But on day 14 the p-value <0,05, meaning that there was a difference between the treatment group and the negative control. This showed that there was an effect of giving binahong extract gel 5% on the number of macrophage cells in the wound healing process after tooth extraction of Wistar strain white rats compared to a negative control group using CMC-Na 1%.

Research on the effect of binahong leaf extract gel (*Anredera cordifolia*) concentration of 5% on the number of macrophages in the wound healing process after tooth extraction of white rats (*Rattus novergicus*) Wistar strain has been carried out using 3 groups, namely the negative control group, positive group, and treatment group. The negative control group was CMC-Na 1%, the positive control group was iod glycerin, and the treatment group was binahong leaf extract gel (*Anredera cordifolia*) concentration of 5%.

The results showed that binahong leaf extract gel (*Anredera cordifolia*) has an influence in accelerating wound healing after tooth extraction by increasing the number of macrophages. This study supports previous research on the effect of binahong leaf extract gel (*Anredera cordifolia*) on osteoblast wounds after tooth extraction of wistar white rats (*Rattus novergicus*) (Sa'diyah *et al.*, 2019).

The results of the study can be seen that there is a difference in the graph of the average number of macrophages between the control group and the treatment group. The average negative control group was high on days 3 and 7, but on days 5 and 14 it decreased. While the average positive control group day 3 to day 7 was higher, but decreased on day 14. The 5% binahong leaf extract gel treatment group had an overview of the highest average macrophage graph on day 7 which increased from day 3 to day 5, then decreased on day 14.

Day 3 post-wound, monocytes will differentiate into macrophages. The number of macrophage cells on day 3 is still small. This is due to the still occurrence of the

inflammatory process on day 3, in the treatment group at least because the inflammatory process is inhibited due to the presence of active compounds from binahong leaves such as flavonoids that can inhibit the inflammatory process in the arachidonic acid pathway and saponin compounds that produce anti-inflammatory cytokines (Izzati, 2015), resulting in the formation of fewer macrophage cells. Macrophages have a role as phagocytosis of foreign objects in the body, producing cytokines, platelet derived growth factor (PDGF), tumor necrosis factor-alpha (TNF- α) to increase the expression of vascular endothelial growth factor (VEGF) (Kumar, 2019).

Day 5 post-wound, the number of macrophages in the negative control group decreased, and the positive control group experienced a slight increase, while the treatment group increased. The LSD test showed that between the treatment group with a positive control group and the treatment group with a negative control group, a p-value of < 0.05 was obtained, which means that there was a significant difference in the number of macrophages in the treatment and control groups. This proves that binahong leaf extract gel has a better ability to stimulate macrophage cells that come to the wound area than Na-CMC 1% and iod glycerin. The cause of the increase in the number of macrophages on day 5 is due to an increase in vascular endothelial growth factor (VEGF). The high number of macrophages on day 5 was affected in the treatment group. Binahong leaves have flavonoid compounds that produce IFN- γ (Interferon- γ) which has the ability to increase the number of macrophages. Flavonoids are also immunostimulators through phagocytosis activity.

Day 7 in the negative control group, positive control group, and treatment group showed an increased macrophage count compared to day 5. The results of the LSD test showed that in the treatment group, the positive control group, and the negative control group p-value $< 0,05$ which can be said to be a significant difference in the number of macrophages. The number of macrophage cells that rose from day 5 in the control group was negative, positive control indicates that the inflammatory process is still occurring. Inflammation can be caused by the remnants of cell debris necrosis in the wound area that has not been phagocytosed optimally the previous day (Kumar *et al.*, 2013). Meanwhile, the treatment group is caused because phagocytosis activity is still increasing because there are flavonoids that can increase IL-2 (Interleukin 2) activity. In addition, the content of saponins in binahong leaf extract is referred to as a compound that is "proangiogenesis" because it can increase the expression of Vascular Endothelial Growth Factor (VEGF), thereby stimulating macrophage cell growth (Fatihatuzzahroh *et al.*, 2015).

Day 14 the number of macrophages showed a drastic decrease in the negative control group, positive control, and treatment group which indicated the inflammation phase was complete and entered the next phase, namely the proliferation phase. This proliferative phase involves M2 phenotypic macrophages, keratinocytes, and treg lymphocytes that can promote re-epithelialization, angiogenesis, and fibroplasia. In addition, macrophages and treg lymphocytes work to inhibit the inflammatory phase so that it immediately changes to the proliferative phase (Wilkinson dan Hardman, 2020).

The treatment group, namely binahong leaves, has effective ingredients for wound healing such as flavonoids, alkaloids, saponins, terpenoids, tannins, and ascorbic acid (Ardiana *et al.*, 2015). The content in binahong leaves is thought to increase the number of macrophages in the wound healing process after tooth extraction. The flavonoid content in binahong leaves is a metabolite compound that has antibacterial activity and a free radical scavenger that has an anti-inflammatory function. The ability of metabolites in flavonoid content in binahong extract can increase the number of macrophages so

that the proliferation phase in wound healing can be started earlier due to the inflammatory phase which is shorter in time compared to normal conditions at the time of wound healing. The number of macrophages can also affect the number of new macrophages. The activity of macrophages in wound healing in the proliferative phase is to express several important growth factors such as Vascular Endothelial Growth Factor (VEGF) which acts as a cytokine in the process of forming new macrophages (Fitrian *et al.*, 2018).

The content of saponins in binahong leaves is a class of steroids that are able to induce angiogenesis by increasing protease activity and endothelial migration (Nugroho *et al.*, 2017). Binahong leaves also contain tannins have a role to stop bleeding, treat infections from wounds, and are able to create a protective layer of wounds (Nurrani *et al.*, 2015).

The content of terpenoids in binahong leaves acts as an antioxidant. The content of alkaloids and ascorbic acid can also accelerate the wound healing process because it contains anti-inflammatory content that can strengthen cell resistance to infection so that cells can regenerate properly. In addition, ascorbic acid is an important factor in the formation of the enzyme prolyl hydroxylase which functions for collagen biosynthesis in the wound healing process (Ravetti *et al.*, 2019). This showed that binahong leaf extract gel (*Anredera cordifolia*) concentration of 5% was effective in accelerating wound healing after tooth extraction by accelerating the decrease in the number of macrophages on day 14 as an indicator that the wound healing process was about to end.

According to research by Rahmanita *et al.* (2023), binahong leaves contain chemical compounds such as: flavonoids, ascorbic acid, tannins, terpenoids, saponins, and alkaloids that can heal wounds. Flavonoids have activity as immunostimulators through the phagocytosis activity of macrophages. The mechanism of flavonoids in macrophage phagocytosis activity is to increase IL-2 (Interleukin 2) activity which results in accelerated macrophage cell proliferation and activity. Tannins and alkaloids have antioxidant and antimicrobial properties that help the wound healing process by preventing and maintaining the wound area from free radical damage and inhibiting the growth of pathogenic bacteria in the wound (Hanafiah *et al.*, 2017).

Terpenoids are compounds that work quickly in inhibiting bacterial mechanisms by dysfunction or rupture of bacterial cell membranes. Saponins work to fight antigens so that they can ease the work of inflammatory cells such as macrophage cells, neutrophil cells, and lymphocyte cells when there are antigens in the body (Sharma *et al.*, 2020). Alkaloids are immunomodulatory just like flavonoid compounds, namely by increasing the production of the cytokine IL-2. IL-2 cytokines can affect CD4⁺ and cause Th1 cells to activate. Activated Th1 cells will affect IFN- γ . This IFN- γ cytokine will activate monocytes into macrophages and then phagocytosis when there are antigens that enter the body (Kotala dan Kurnia, 2022).

LSD test results on days 3, 5, 7, and 14 between the treatment group and the positive control group had a p-value of <0,05, indicating a significant difference between the two groups. The negative control group had a p-value of <0,05 on day 5, but seen from Figure 4 on day 3, and day 7 showed an increase in the number of macrophage cells, while on day 5 and day 14 showed a decrease in the number of macrophage cells, this is due to the nature of CMC-Na 1% which is a neutral gel base which means it does not have an effect or change the properties of other active compounds on the healing process so it does not can affect the increase in the number of macrophages in the wound healing process (Puspitasary *et al.*, 2019).

Another factor that accelerates wound healing is binahong leaf extract which is

packaged in gel form, so that this gel dosage form is able to maintain wound moisture in accordance with the principle of moist wound healing. Moist wound conditions will stimulate the formation of growth factors by macrophages such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and thrombospondin which will stimulate endothelial cells (Nugroho *et al.*, 2017).

CONCLUSIONS

It can be concluded that the administration of binahong leaf extract gel (*Anredera cordifolia*) concentration of 5% had an effect on increasing the number of macrophages in the wound healing process after tooth extraction of white rats (*Rattus norvegicus*) wistar strains.

REFERENCES

- Al-Hajj NQM, Algabr M, Sharif HR, Aboshora W, Wang H. 2016. In Vitro and in Vivo Evaluation of Antidiabetic Activity of Leaf Essential Oil of *Pulicaria inuloides*-Asteraceae. *Journal of Food and Nutrition Research*, 4(7): 461–470. <https://pubs.sciepub.com/jfnr/4/7/8/index.html>
- Angria N. 2019. Undur-Undur (*Myrmeleon sp.*) sebagai Antidiabetik. Ponorogo : Uwais Inspirasi Indonesia.
- Anggraini M. 2016. Pengaruh Konsentrasi Carboxy Methyl Cellulose (CMC) dan Lama Penyimpanan pada Suhu Dingin terhadap Stabilitas dan Karakteristik Minuman Probiotik Sari Buah Nanas. [skripsi]. Bandar Lampung: Universitas Lampung.
- Ani RP, Usman, Fauzan S. 2018. Pengaruh Pemberian Kompres Jahe Merah (*Zingiber Officinale Var Rubrum Rhizoma*) terhadap Nyeri pada Pasien Gout Arthritis di Wilayah Kerja Puskesmas Aliyayang Kota Pontianak.[skripsi]. Pontianak: Universitas Tanjungpura.
- Ardiana T, Kusuma ARP, Firdausy MD. 2015. Efektivitas Pemberian Gel Binahong (*Anredera cordifolia*) 5% terhadap Jumlah Sel Fibroblast pada Soket Pasca Pencabutan Gigi Marmut (*Cavia cobaya*). *Odonto Dental Journal*, 2(1): 64-70. <https://jurnal.unissula.ac.id/index.php/odj/article/view/440/367>
- Arief H, Widodo MA. 2016. Peranan Stres Oksidatif pada Proses Penyembuhan Luka. *Jurnal Ilmiah Kedokteran Wijaya Kusuma*, 5(2): 22-29. <http://dx.doi.org/10.30742/jikw.v5i2.338>
- Budi HS, Soesilowati P, Imanima Z. 2017. Gambaran Histopatologi Penyembuhan Luka Pencabutan Gigi pada Makrofag dan Neovascular dengan Pemberian Getah Batang Pisang Ambon. *Majalah Kedokteran Gigi Indonesia*, 3(3): 121-127. <https://repository.unair.ac.id/111025/>
- Fatihuzzahroh, Firani NK, Kristianto, H. 2015. Efektifitas Ekstrak Bunga Cengkeh (*Syzygium aromaticum*) terhadap Jumlah Pembuluh Darah Kapiler pada Proses Penyembuhan Luka Insisi Fase Proliferasi. *Majalah Kesehatan*, 2(2): 92-98. https://scholar.google.co.id/citations?view_op=view_citation&hl=id&user=7FwnZqkAAAAJ&citation_for_view=7FwnZqkAAAAJ:Tyk-4Ss8FVUC
- Fitrian A, Bashori A, Sudiana L. 2018. Efek Angiogenesis Gel Ekstrak Daun Lamtoro (*Leucaena Leucocephala*) pada Luka Insisi Tikus. *Jurnal Biosains Pascasarjana*, 20: 22-32. <https://doi.org/10.20473/jbp.v20i1.2018.22-32>
- Ghlichloo I, Gerriets V. 2023. Nonsteroid Anti-Inflammatory Drugs (NSAIDs).

- National Library of Medicine.
- Hanafiah OA, Hanafiah DS, Dohude GA, Satria D, Livita, Moudy NS, *et al.* 2021. Effects of 3% Binahong (*Anredera cordifolia*) Leaf Extract Gel on Alveolar Bone Healing in Post-Extraction Tooth Socket Wound in Wistar Rats (*Rattus Norvegicus*). F1000Research, 10: 1-23. <https://doi.org/10.12688/f1000research.72982.2>
- Hanafiah OA, Hanafiah DS, Bayu ES, Abidin T. 2017. Quantity Differences of Secondary Metabolites (Saponins, Tannins, and Flavonoids) from Binahong Plant Extract (*Anredera Cordifolia* (Ten.) Steenis) Treated and Untreated with Colchicines That Play a Role in Wound Healing. World Journal of Dentistry, 8(4): 296–299. <http://dx.doi.org/10.5005/jp-journals-10015-1453>
- Izzati UZ. 2015. Efektivitas Penyembuhan Luka Bakar Salep Ekstrak Etanol Daun Senggani (*Melastoma malabathricum L*) pada Tikus (*Rattus norvegicus*) Jantan Galur Wistar. Jurnal Mahasiswa Farmasi Fakultas Kedokteran UNTAN, 3(1): 1-19. <https://jurnal.untan.ac.id/index.php/jmfarmasi/article/view/11025/10497>
- Koraag JR, Leman MA, Siagian KV. 2015. Efektivitas Perasan Daun Pepaya terhadap Jumlah Osteoblas Pasca Pencabutan Gigi pada Tikus Wistar Jantan. Pharmacon: Jurnal Ilmiah Farmasi Unsrat, 4(4):40–46. <https://ejournal.unsrat.ac.id/v3/index.php/pharmacon/article/view/10190>
- Kotala S, Kurnia TS. 2022. Eksplorasi Tumbuhan Obat Berpotensi Imunomodulator di Kecamatan Leihitu Kabupaten Maluku Tengah. Sainmatika: Jurnal Ilmiah Matematika dan Ilmu Pengetahuan Alam, 19(2): 186–200. <https://doi.org/10.31851/sainmatika.v19i2.9508>
- Kresnadi E. 2013. Stress Hiperglikemia. Jurnal Kedokteran Universitas Mataram, 2(3): 51–60. <https://jku.unram.ac.id/index.php/jk/article/view/70>
- Kumar V, Abbas AK, Aster JC. 2013. Robbins Basic Pathology Edisi 9. Canada: Elsevier Saunders.
- Kumar V. 2019. Macrophages: The Potent Immunoregulatory Innate Immune Cell. Biology and Disease, 1-30. <https://doi.org/10.5772/intechopen.88013>
- Kurniawati H, Puri KE, Aziz A. 2024. Aksi Nyata Sehatkan Gigi dan Mulut Masyarakat Guna Mewujudkan Indonesia Bebas Karies 2030 di SDN 1 Sandik Lombok Barat. Jurnal Pengabdian Magister Pendidikan IPA, 7(1): 355-357. <https://jppipa.unram.ac.id/index.php/jpmpi/article/view/8449>
- Landén NX, Li D, Stähle M. 2016. Transition from Inflammation to Proliferation: A Critical Step during Wound Healing. Cellular and Molecular Life Sciences, 73(20): 3861–3885. <https://doi.org/10.1007/s00018-016-2268-0>
- Latief M, Tarigan IL, Sari PM, Aurora FE. 2021. Aktivitas Antihiperurisemia Ekstrak Etanol Daun Sungkai (*Peronema canescens Jack*) pada Mencit Putih Jantan. Pharmacon: Jurnal Farmasi Indonesia, 18(1): 23–37. https://www.academia.edu/69385446/Aktivitas_Antihiperurisemia_Ekstrak_Etanol_Daun_Sungkai_Peronema_canescens_Jack_Pada_Mencit_Putih_Jantan
- Mokondongan AR, Nangoy F, Leke JR, Poli Z. 2017. Penampilan Pertumbuhan Ayam Bangkok Starter yang Diberi Pakan dengan Level Protein Berbeda. Jurnal Zootek, 37(2): 426-435. <https://doi.org/10.35792/zot.37.2.2017.16268>
- Nugrahwati F. 2016. Uji Aktivitas Antipiretik Ekstrak Daun Bidara (*Ziziphus sphinachristi l.*) terhadap Mencit Jantan (*Mus musculus*). [skripsi]. Makassar: Universitas Islam Negeri Alauddin.
- Nugroho AM, Elfiah U, Normasari R. 2017. Pengaruh Gel Ekstrak dan Serbuk Mentimun (*Cucumis sativus*) terhadap Angiogenesis pada Penyembuhan Luka

- Bakar Derajat IIB pada Tikus Wistar, 4(3): 443-448. <http://repository.unej.ac.id/handle/123456789/82560>
- Nurrani L, Tabba S, Mokodompit HS. 2015. Kearifan Lokal dalam Pemanfaatan Tumbuhan Obat oleh Masyarakat di sekitar Taman Nasional Aketajawe Lobata, Provinsi Maluku Utara. *Jurnal Penelitian Sosial dan Ekonomi Kehutanan*, 12(3): 163-175. <https://www.neliti.com/publications/29178/kearifan-lokal-dalam-pemanfaatan-tumbuhan-obat-oleh-masyarakat-di-sekitar-taman>
- Prameswari AK, Utari D, Hardy FR, Fitri AM. 2022. Tingkat Kelelahan Pekerja pada Masa Pandemi Covid-19 di Proyek Pembangunan Ruas Jalan Tol Serpong-Cinere. *Jurnal Kesehatan Komunitas*, 8(3): 562-570. <http://dx.doi.org/10.25311/keskom.Vol8.Iss3.858>
- Primidina N, Basori A, Perdanakusuma DS. 2019. Proses Penyembuhan Luka Ditinjau dari Aspek Mekanisme Seluler dan Molekuler. *Qanun Medika: Jurnal Kedokteran FKUM Surabaya*, 3(1), 31-43. <https://journal.um-surabaya.ac.id/qanunmedika/article/view/2198>
- Purnama H, Sriwidodo, Mita RS. 2017. Proses Penyembuhan dan Perawatan Luka: Review Sistematis. *Farmaka*, 15(2): 251-258. <https://jurnal.unpad.ac.id/farmaka/article/view/13366>
- Puspitasary K, Novitasari M, Widyaningrum NR. 2019. Pengaruh Perbandingan Sodium Carboxy Methyl Cellulose (CMC Na) terhadap Uji Fisik Gel Ekstrak Daun Teh Hijau (*Camelia Sinensis L.*). *Avicenna: Journal of Health Research*, 2(2): 111-120. <https://doi.org/10.36419/avicenna.v2i2.307>
- Rahmanita E, Negara YDP, Kustiyahningsih Y, Sasmeka V, Khotimah BK. 2023. Implementasi Metode Naïve Bayes dan Information Gain untuk Klasifikasi Penyakit dan Hama Tanaman Jagung. *Teknika: Jurnal Teknologi Informasi dan Komunikasi*, 12(3): 198-204. <https://doi.org/10.34148/teknika.v12i3.684>
- Ravetti S, Clemente C, Brignone S, Hergert L, Allemandi D, Palma S. 2019. Ascorbic Acid in Health Skin. *Cosmetics*, 6(4): 1-8. <https://doi.org/10.3390/cosmetics6040058>
- Sa'diyah JS, Septiana DA, Farih NN, Ningsih JR. 2019. Pengaruh Gel Ekstrak Daun Binahong (*Anredera cordifolia*) Konsentrasi 5% terhadap Re-epitelisasi Luka Pasca Pencabutan Gigi Tikus Putih Wistar (*Rattus Norvegicus*). *Jurnal Kedokteran Gigi Universitas Padjadjaran*, 31(3): 233-238. <https://jurnal.unpad.ac.id/jkg/article/view/21833>
- Sharma R, Palanisamy A, Dhama K, Mal G, Singh B, Singh KP. 2020. Exploring the Possible use of Saponin Adjuvants in Covid-19 Vaccine. *Human Vaccines and Immunotherapeutics*, 16(12): 2944-2953. <https://doi.org/10.1080/21645515.2020.1833579>
- Sitanaya RI. 2016. *Exodontia: Dasar-Dasar Ilmu Pencabutan Gigi*. Yogyakarta: Deepublish.
- Susetya D. 2016. *Panduan Lengkap Membuat Pupuk Organik: untuk Tanaman Pertanian dan Perkebunan*. Yogyakarta: Pustaka Baru Press.
- Wilkinson HN, Hardman MJ. 2020. Wound Healing: Cellular mechanisms and Pathological Outcomes. *Open Biology Journal*, 10: 1-14. <https://doi.org/10.1098/rsob.200223>
- Yuniarti WM, Lukiswanto BS. 2017. Effect of Herbal Ointment Containing the Leaf Extracts of Madeira Vine (*Anredera cordifolia* (Ten.) Steenis) for Burn Wound Healing Process on Albino Rats. *Veterinary World*, 10(7): 808-813. <https://www.veterinaryworld.org/Vol.10/July-2017/17.html>