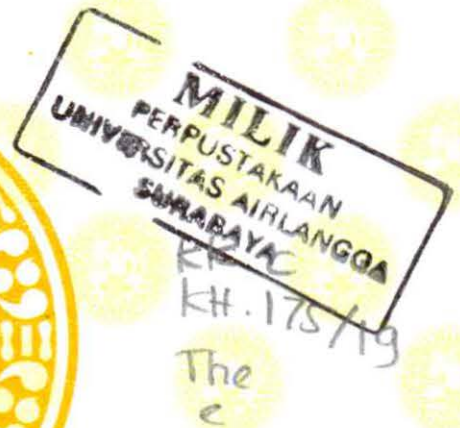


THESIS

**THE EFFECT OF MADEIRA VINE LEAF EXTRACT
(*Anredera cordifolia* (Ten.) steenis) COMBINED WITH
TURMERIC RHIZOME EXTRACT (*Curcuma longa*)
GEL GIVEN TOPICALLY TO IIB BURN WOUND
OF WHITE RAT (*Rattus norvegicus*)
ON FIBROBLAST NUMBER**



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**FACULTY OF VETERINARY MEDICINE
UNIVERSITAS AIRLANGGA
SURABAYA
2019**

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ON FIBROBLAST NUMBER**

Thesis
Submitted in partial fulfillment of the requirement for the degree of
Bachelor Veterinary Medicine
at
Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya

By :

SIS WISNUARTI THERESIA
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DECLARATION

Hereby, I declare that in the thesis entitled:

The Effect of Madeira Vine Leaf Extract (*Anredera cordifolia* (Ten.) *steenis*) Combined with Turmeric Rhizome Extract (*Curcuma longa*) Gel Given Topically to IIB Burn Wound of White Rat (*Rattus norvegicus*) On Fibroblast Number

there is no other work ever published to obtain bachelor degree in any other universities before and as I have ever known that there are no papers or opinions which have been written or published by somebody else, besides the writing which is referred in this paper and mentioned in the references.



Surabaya, 8th July 2019



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SUMMARY

SIS WISNUARTI THERESIA. The effect of Madeira vine leaf extract (*Anredera cordifolia* (Ten.) *steenis*) combined with 2% Turmeric rhizome extract (*Curcuma longa*) gel given topically to II B burn wound of white rat (*Rattus norvegicus*) on the number of fibroblast. Outcome of this research is to see the effect of giving Madeira vine leaf extract combined with 2% Turmeric rhizome extract gel in increasing number of fibroblasts in healing process of II B burn wound on white rat compared with SSD ointment. Good healing process is indicated by fibroblast and collagen. Fibroblast synthesize collagen to make new tissue framework. The more fibroblast the more collagen formed and it makes stronger new tissue framework.

Madeira vine leaf extract contain saponin, flavonoid, tannin, vitamin C, and oleanic acid that have potential to speed up the healing process because they have anti-inflammation, antioxidant, and anti-bacterial. Turmeric rhizome extract contain curcumin that works as anti-inflammation, anti-oxidants, anti-microbial, and also known to stimulate TGF- β 1 that play a role in fibroblast proliferation. Gel base used in this extract to soothe the burn wound because its ability to cool down the burn wound and reduce the pain.

This research used 25 male white rats, Wistar strain, weight about 200-300 gram. They are divided into 5 treatment groups, such as C- (normal healthy skin), C+ (burn wound given with 1% SSD), T1 (burn wound with 1.25% MVLTR gel), T2 (burn wound with 2.5% MVLTR gel), and T3 (burn wound with 5% MVLTR

gel). Burns were made with modified thermostat at 85°C for 5 seconds. Burns treatment were given twice a day for 14 days. Samples were taken on the 15th day.

Skin samples were made into histology slide using HE stain. Histology slide were observed using Optilab and Nikon 600L microscope at 100x magnification to determine the healing center first, then using 400x magnification to calculated the number of fibroblast with Image Raster application in five field of views. The average data of fibroblast number was analyzed using SPSS 20.0 with One Way ANOVA and the Duncan method to see significant value between treatments with significant differences value $p < 0.05$.

Result of this research showed that Madeira leaf extract combined with 2% Turmeric rhizome extract gel could increase the fibroblast number. Based on the data the highest number of fibroblast was found in T3 (5% MVLTR). T1 (1.25% MVLTR) was not recommended for clinical use because it showed lower fibroblast number than C+ (SSD ointment). Both T2 (2.5% MVLTR) and T3 (5% MVLTR) showed higher fibroblast number compare to C+ (SSD ointment) and were not significantly different from the data. Therefore, T3 was showed the highest fibroblast number and that makes 5% concentration of MVLTR gel as the most effective treatment and optimal dose to be used as burn wound treatment in this study. The result of this study also showed that Madeira vine leaf extract combined with 2% Turmeric extract gel with specific concentration (5% suggested) can be use as replacement of Silver Sulfadiazine ointment in burns treatment, especially in deep second degree burn wound.

THE EFFECT OF MADEIRA VINE LEAF EXTRACT (*Anredera cordifolia* (Ten.) *steenis*) COMBINED WITH TURMERIC RHIZOME EXTRACT (*Curcuma longa*) GEL GIVEN TOPICALLY TO IIB BURN WOUND OF WHITE RAT (*Rattus norvegicus*) ON FIBROBLAST NUMBER

Sis Wisnuarti Theresia



ABSTRACT

The aim of this study was to determine the effect of Madeira vine (*Anredera cordifolia* (Ten.) *steenis*) leaf extract combined with 2% Turmeric (*Curcuma longa*) rhizome extract (MVLTR) gel given topically to IIB burn wound of rat (*Rattus norvegicus*) on the number of fibroblast. 25 male rats with 200-250 gram body weight were divided into five groups. C- group was normal skin, C+ group was skin burn treated with 1% Silver Sulfadiazine, T1 was skin burn treated with 1.25% MVLTR gel, T2 was skin burn treated with 2.5% MVLTR gel, and T3 was skin burn treated with 5% MVLTR gel. The burn wound was made on the right gluteus of rats and the therapy had been given topically twice a day, started at the time after burn wounds were made. Skin samples were collected after 14 days of treatment. The histopathological examination for the number of fibroblast was performed by counting fibroblast cells then analyzed with One Way ANOVA and continued with Duncan test. MVLTR gel at 5% concentration has potential to be an effective treatment for IIB burns as indicated by increased number of fibroblast because MVLTR have antibacterial, anti-inflammatory and antioxidant effects during the burn wound healing process. Saponin, flavonoid, and tannin in Madeira vine leaf extract and curcumin in Turmeric rhizome extract accelerated fibroblast proliferation. Therefore, this study concluded that 5% MVLTR gel is the effective dosage to increase the number of fibroblast on deep second degree burn wound healing in rat.

Keywords: *Anredera cordifolia*, *Curcuma longa*, fibroblast, burn wound healing

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ABBREVIATION AND MEANING SYMBOL

ANOVA	: <i>Analysis of Variance</i>
CMC	: <i>Carboxy Methyl Cellulose</i>
COX	: Cyclooxygenase
ECM	: Extracellular Matrix
EGF	: Epithelial Growth Factor
Et al.	: <i>et alli</i>
FGF	: Fibroblast Growth Factor
HE	: Hematoxylin and Eosin
IL	: Interleukin
iNOS	: inducible Nitric Oxide Synthase
MVLTR	: Madeira Vine leaf extract combined with Turmeric rhizome extract
PDGF	: Platelet-Derived Growth Factor
ROS	: Reactive Oxygen Species
SSD	: <i>Silver Sulfadiazine</i>
TBSA	: <i>Total Body Surface Area</i>
TGF	: <i>Transforming Growth Factor</i>
TNF	: <i>Tumor Necrosis Factor</i>
WHO	: World Health Organization

CHAPTER 1

INTRODUCTION



CHAPTER I INTRODUCTION

1.1 Research Background

Burns are now considered to be one of the most destructive forms of trauma that affect humans and animals. According to World Health Organization (WHO) Global Burden Disease estimates for 2017, just over 180.000 people died from burns and more than 2,5 million people in America got wound from it. Burn wound is an injury to the part of the body, mostly to the skin, that caused by thermal trauma. There are various causes of thermal trauma, some of them including fire, hot liquids, hot solids, radiations, chemicals, and electricity. The most common cases of burn wound in general come from hot liquids and flame burn (Atalay *et al.*, 2014).

Burn wounds can be classified in several ways, but mostly they're classified according to depth and thickness of the damage skin. There are first-degree burns which also known as epithelial burns, second-degree burns which divided into two types the superficial second-degree (II A) burn and the deep second-degree (II B) burn, and last is third-degree burns (Tiwari, 2012). Second-degree burn is the most common burn wound case that happen in general population. Second-degree burn will damage the epidermis layer through the dermis layer of the skin. The healing time for the second-degree burn takes around two until three weeks to close and form a scar (Wardhana, 2014).

Skin is the body's natural barrier to infection so if it damaged the healing process will start. Wound healing processes are inflammation, proliferation and remodeling of the tissue. Those process are considered as a complex process in burn injuries

(Mehrabani *et al.*, 2015). One of the important components in the wound healing phase is the fibroblast proliferation. Fibroblast proliferation will effect the formation of collagen, which needed as the framework of new tissue (Sumbayak, 2015; Yuniarti and Lukiswanto, 2017).

Burn wound could happen anytime to anyone and any animals and when it happens it should be treated in the right way. The usual first treatments for burn wound are wound cleansing, debridement (process to removes dead, damaged, or infectious tissue), application of local antibiotic, bandage, and preparation to do skin graft if needed. After the first treatments, wound care is the next step. Wound care for burn wound usually using the silver sulfadiazine (SSD) cream. SSD has been used clinically as a standard treatment for burns over the past three decades and works as the topical antimicrobial agents to prevent contamination on burns (Cooper *et al.*, 1991; McCauley *et al.*, 1994).

The silver element of SSD was shown to be highly toxic to both keratinocytes and fibroblasts (Poon and Burd, 2004). The conventional anti-infective property of SSD can not provide the moisture to boost a rapid wound healing (Homann *et al.*, 2007). These condition triggered the population to seek for the new treatment with the less side-effect and better efficacy.

Traditional herbal medicine always become the interesting answer when it comes to finding the new alternative from the chemical medicine. Traditional herbal medicine has been used since hundred years ago with our ancient as the cure for a lot of diseases. The World Health Organization (WHO) has recently defined traditional herbal medicine as one of therapeutic practice that have been in

existence. Some of the herbal products has been tested and they showed a moderate efficacy with no or less toxicity.

Traditional herbal medicines had been developed and used for healing of wounds for decades in the world of medicine. Herbal plants and herbs have been widely used in wound healing of burned injuries (Karimi *et al.*, 2013). To help speed up the healing process of wounds, the herbal plants and herbs usually have saponins, flavonoids, tannins, and alkaloids as the active substances (Astuti, 2011). However, clinical experiment to prove the exact effect of them are still minim.

One of the herbal plants that contain most of active substances that mention above is Madeira vine, known as Binahong in Indonesia. This plant has been growing very well since long time ago and in the society in Java it is trusted as the miracle plant because this plant can be use as the treatment for various diseases beside wound healing, such as diabetes mellitus, hypertension, rheumatics, typhoid fever, tuberculosis, asthma and many more. However, its capability is still not well documented (Hanafiah, 2017 and Astuti, *et al.*, 2011). Most part that been used in scientific experiments is leaves.

Madeira vine leaf contains active substances such as flavonoid, saponin, tannin, and alkaloids. The flavonoid in the leaves of Madeira vine has the potential as antioxidant, antibacterial, anti-aging, anti-leukemic, and vasodilator activity, while saponin works to stimulate the formation of collagen by increasing the number of fibroblast and also works as an antiseptic that can terminate or prevent the growth of microorganism in the wound to avoid an infection. Tannin and alkaloids have antioxidants and antimicrobial properties that prevent and keep the wound area

contaminated keeping the wound area from being damaged by free radicals and inhibit the growth of pathogenic bacteria in the wound (Suratman *et al.*, 1996 and Sasidharan *et al.*, 2010).

One of the herbal herbs that had been reviewed to help speed up wound healing process is Turmeric. Turmeric has been used as a spice, in medicine, and for coloring and flavoring food since ancient times in Asian countries. Rhizome part is the usual part of Turmeric to be use in food and medicine. Researchers have found a natural product that believed to be the key substance from Turmeric and it comes from the rhizomes, it known as Curcumin (Hewlings and Kalman, 2017; Jovičić *et al.*, 2017; Li *et al.*, 2012).

Various studies have also shown that Curcumin's application on wound enhances epithelial regeneration and increases fibroblast proliferation and vascular density (Sidhu *et al.*, 1998; Thangapazham *et al.*, 2013). Despite its reported benefits, there is still one of the major problems with using Curcumin by itself is its poor bioavailability, the poor water solubility as well as instability, had greatly limited its further applications (Anand *et al.*, 2007 and Li *et al.*, 2012).

Traditional herbal medicine will be easier to use with the gel base because it has high level of water. Gel base have shorter deployment time which will prevent the infection of the wounds. The gel base could also help Curcumin to have a better bioavailability and help its applications on wounds. Both Madeira vine leaves and Turmeric's rhizome present substances that have the ability to speed up wound healing process, however Binahong leaf is more common to be use in burn treatment and Turmeric rhizome is more common to be use in wound treatment.

Based on this research background, author is interested to conduct research of the effect of applying gel of Madeira vine leaves (*Anredera cordifolia* (Ten.) steenis) extract combined with Turmeric rhizome (*Curcuma longa*) extract to the number of fibroblast as one of the wound healing factors on deep second-degree (II B) burn wound.

1.2 Problem Statement

Based on the research background, problem statement from this research is By applying gel of Madeira vine leaf extract (*Anredera cordifolia* (Ten.) steenis) combined with Turmeric rhizome extract (*Curcuma longa*) topically to deep second-degree burn wound of white rat (*Rattus norvegicus*) could it increase the number of fibroblast?

1.3 Theoretical Base

Wound can heal naturally, however there are some factors that have role in the process such as age, oxygen free radicals, malnutrition, etc (Paramita, 2016; Parihar, 2008). Wound healing process in general consist of three phases such as inflammatory, proliferation, and remodeling. The proliferative phase is characterized by keratinocyte and fibroblast activation by cytokines and growth factors (Werner et al., 2017). Fibroblasts are cells that are responsible to form collagen which is one of the key components in wound healing process (Porter, 2007). This shows fibroblast as an important part in wound healing process.

Some of the Madeira Vine leaf component such as saponin, tannin, and flavonoid are known to have the ability to the migration and proliferation of

fibroblasts, increase the number of fibroblast cells, stimulate the formation of collagen and also as an antioxidant (Ashok and Upadhyaya, 2012; Kimura *et al.*, 2010; Sasidharan *et al.*, 2010). Curcumin, the component from Turmeric rhizome, is also known to have the ability to increase fibroblast proliferation and vascular density (Thangapazham *et al.*, 2013). Curcumin is also one of the natural antioxidant, most potent anti-inflammatory antioxidant in clinical medicine (Panjeshahin *et al.*, 2003). The ideal application form for the burn wound is topical form (Singer *et al.*, 2007).

Research by Widya (2013) proves that flavonoid component in Madeira Vine leaf extract may help in the wound healing process with promotes the formation of collagen and increase the number of fibroblast (Ambiga *et al.*, 2007). Research by Mehrabani *et al.*, 2015 proved that curcumin played a prominent role in wound healing process in burns by the increased migration of myofibroblast, fibroblasts, and macrophages. Curcumin also increased the expression of proliferating cell nuclear antigen in skin tissues of the burn wounds (Kulac *et al.*, 2013).

1.4 Research Aim

This research aim is to know the effect of Madeira vine leaf extract (*Anredera cordifolia* (Ten.) steenis) combined with Turmeric rhizome extract (*Curcuma longa*) gel given topically to deep second-degree burn wound of white rat (*Rattus norvegicus*) on the number of fibroblast.

1.5 Research Outcome

Theoretical Outcome

This research could be further theoretical basis for the development of research into the effect of Madeira vine leaf extract (*Anredera cordifolia* (Ten.) steenis) combined with Turmeric rhizome extract (*Curcuma longa*) gel given topically to deep second-degree burn wound of white rat (*Rattus norvegicus*) on the number of fibroblast.

Practical Outcome

This research would have practical benefits in the development of traditional herbal medicine and to find another alternative medicine for deep second-degree burn wound.

1.6 Research Hypothesis

Based on the problem statement and theoretical base, the hypothesis of this research is the administration of Madeira vine leaf extract (*Anredera cordifolia* (Ten.) steenis) combined with Turmeric rhizome extract (*Curcuma longa*) gel which given topically to deep second-degree burn wound of white rat (*Rattus norvegicus*) in 14 days can help to speed up the healing process, especially to show the difference in increase number of fibroblast between the gel extract and Silver Sulfadiazine.

CHAPTER 2

LITERATURE REVIEW



CHAPTER 2 LITERATURE REVIEW

2.1 Skin Structure

The integumentary system is formed by the skin and its derivative structures (**Figure 2.1**). Skin is the largest organ on the body and has about 15% of the total adult body weight. It has role to be the first protection for every structure above it from any kind of injury and microorganism. It also has role to prevent excess water loss from the body and a role in thermoregulation. There are two major layer in skin, epidermis and dermis. And also subcutaneous tissue, the last layer of skin, as the boundary between skin and any structure above it (Kanitakis, 2002; James *et al.*, 2006; Grant and Waugh, 2014).

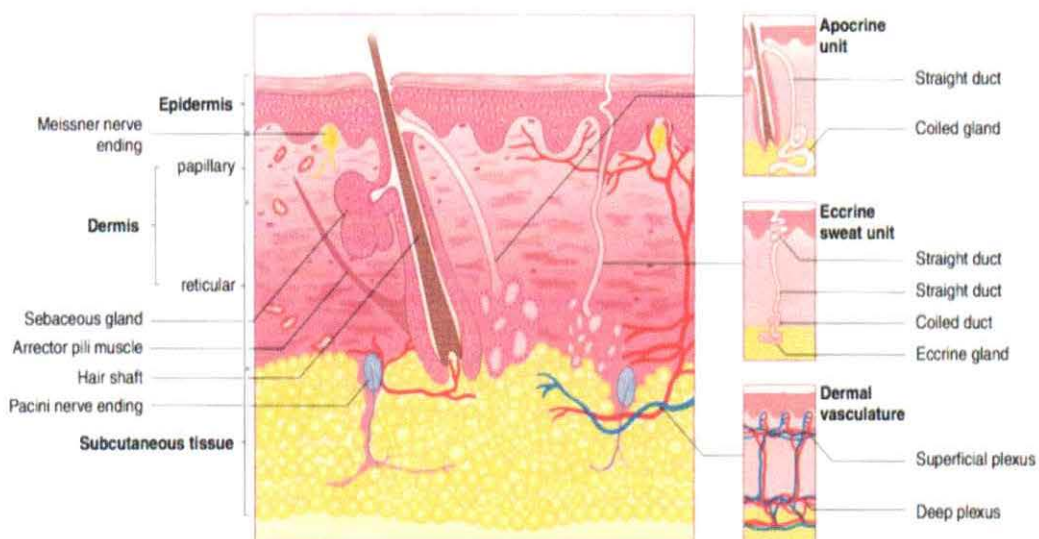


Figure 2.1. Structure of skin (James *et al.*, 2006)

2.1.1 Epidermis

The superficial layer from skin called epidermis. Epidermis consist of stratified, squamous epithelium layer that is composed primarily of two types of cells:

keratinocytes and dendritic cells. This layer has no vascularization or nerve endings, but the interface between the epidermis and dermis is formed by a porous basement membrane zone that allows oxygen and nutrients went to epidermis and the exchange of cells and fluid and holds the two layers together (Grant and Waugh, 2014; James *et al.*, 2006). Epidermis has various thickness depend on the position and the function. The thickest part of epidermis is in palm and the sole of foot. To maintenance a constant epidermal thickness, it needs to undergo apoptosis, programmed cell death (Grant and Waugh, 2014; Kolarsick *et al.*, 2011).

Epidermis commonly is divided into four layers such as the basal cell layer (stratum germinativum), the squamous cell layer (stratum spinosum), the granular cell layer (stratum granulosum), and the cornified cell layer (stratum corneum) (James *et al.*, 2006). Stratum basal consists of one layer of cells arranged in row above the basement membrane and attached to the large core epidermis and basophilic cytoplasm. Stratum spinosum is consist of several layer of polygonal cells with oval nuclei. Stratum granulosum is consisting of 2 - 4 layers of basophilic flatbed granular cells. Stratum corneum is consists of dead cells, flat and not nucleated, the cytoplasm is replaced by keratin (Widyarini, 2016).

Blister happened when trauma caused epidermis and dermis, also serous fluid between both layers detached. Total epidermis alteration occurred for about 1 month, it depends on the damage level (Grant and Waugh, 2014).

2.1.2 Dermis

Dermis layer is elastic and solid and consists of connective tissue derived from the mesoderm which functions as connective tissue to support the epidermis and matrix that contain collagen which intertwined with elastic fibers. Dermis is an integrated system of fibrous, filamentous, and loose connective tissue that accommodates stimulus-induced entry by nerve and vascular networks, fibroblasts, macrophages, and mast cells. Fibroblast, mast cell, and macrophages are the main cells that found in dermis. Many fibroblasts are present in the infant dermis, but few persist in adulthood (James *et al.*, 2006)

Dermis consists of two layers with unclear boundaries, in the outer layer is stratum papillary and the inner layer is stratum reticular. In stratum papillary there is a broad capillary network that surrounds the epidermis and has several functions such as regulating body temperature, and helping the epidermis that does not have blood vessels to get nutrients. Stratum reticular is thicker and is composed of irregular solid connective tissue (Grant and Waugh, 2014; Hestianah *et al.*, 2014).

Dermis interacts with epidermis to maintain contents of both layers. Both dermis and epidermis collaborate during development in the morphogenesis of the dermal-epidermal junction and in repairing and remodeling the skin as wounds are healed (Kolarsick *et al.*, 2011).

2.2 Burn Wound

Burn wound is skin damaged that caused by exposure to heat (thermal burns), electricity, chemicals, radiation, flame, hot liquid, chemicals, or electricity to the body (Ghofar and Mawarti, 2014). Scalding (hot liquids) and flame burns are the

most common causes of burns in general population (Atalay *et al.*, 2014). This skin damage requires special care because it caused high morbidity and mortality that can affect local and systemic changes in the body. The depth of tissue damage due to burns depends on the source, type, and duration of contact with the body surface (Anggowarsito, 2014).

Thermal injury effect in the disruption of cell membrane integrity, activation of cytokines, and discontinuance of local blood flow (Orgill *et al.*, 2005). The severity of a thermal injury depends on the temperature of the heat source, the size of the affected area, and the duration of contact.

2.2.1 Burn wound pathophysiology

Local reaction

According to research that conducted by Jackson (1953), burns zone in skin divided into three zone, such as coagulation zone, stasis zone, and hyperemia zone (Figure 2.2).

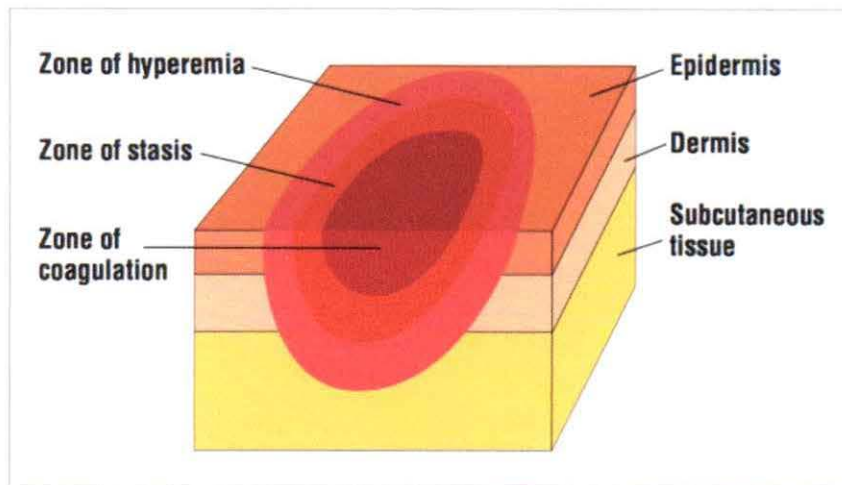


Figure 2.2. Burn model (Jackson's, 1953)

Zone of Coagulation (Necrosis) is the nearest area to the heat source where coagulation of cellular proteins occurs because heat can not be condemned quickly, causing rapid cell death. Often referred to as the center of dead tissue (Roth and Hughes, 2004). This zone also suffers the most damage, sustaining blood clotting and thrombosis of vessels (Frantz and Byers, 2011).

Zone of Stasis is the area that surrounds the coagulation zone and is characterized by a decrease in blood flow (blood stasis) (Frantz and Byers, 2011). If its not being handle immediately this zone will start to undergo necrosis because of the inflammation reaction (Wardhana, 2014).

Zone of Hyperemia is the peripheral area around the burn and is characterized by increased blood flow. The tissue in this zone is likely to recover as long as sepsis or prolonged hypotension do not happen (Frantz and Byers, 2011).

Systemic reaction

When the burn injuries affect more than 30% of total body surface area (TBSA), the release of cytokines and other inflammatory mediators at the site of the injury has a big systemic effect (Hettiaratchy and Dziewulski, 2004). Physiology changes that usually happen in burn wound patients are fluid and electrolyte balance, nerves system, cardiovascular system, respiration system, urinate system, digestion system, immune system, and metabolism system.

2.2.2 Burn wound classification

Burn wounds can be classified according to involvement of skin and deeper tissues into three degrees. First degree only affects epidermis (sunburn), second superficial degree affect epidermis and papillary dermis, second deep degree affect epidermis and reticular dermis, and third degree also known as total thickness affect the three layers of skin and muscle (**Table 2.1**) (Jackson, 1953; Garcia-Espinoza, 2017).

Table 2.1. Clinical characteristics according to the degree of depth

Type of burn	Clinical appearance
First grade	Painful
	Pores the pressure
	Does not leave scar
	Heal 3-6 days
Second grade superficial	Pain, erythema, Flictenas
	Pale to pressure
	Heal in 7-20 days
Deep second grade	Include the reticular dermis
	Whitish, mottled, not paling under pressure
	Heal 2 to 5 weeks with large scar
Third grade	Hard, painless scalps
	Always require grafts

Source: Garcia-Espinoza, 2017

First degree burns

First degree burns are only affect the epidermis layer and the physical characteristic of this burns degree are hyperemic skin in the form of erythema. Its usually cause by sunburn and feel a bit painful because sensory nerve endings are

irritated. Healing process occur spontaneously for about 7 days. This degree is not included in %TBSA calculation (Hidayat, 2013; Wardhana, 2014).

Second superficial degree burns

Second superficial degree also known as superficial partial thickness burns are affect epidermis and papillary dermis. Inflammatory reaction accompanied by exudation process occurs in this degree. Blisters and pinkish color of the wound area are the main physical characteristics of this burns degree. Healing process occur spontaneously for about 14 days with epithelialization of the adnexal structure and might leave scars (Hidayat, 2013; Wardhana, 2014).

Second deep degree burns

Second deep degree also known as deep partial thickness burns are affect epidermis and reticular dermis leaving a bit of epithelial tissue and a few skin organs. Capillary refill is not found in this degree (Wardhana, 2014). The physical characteristics that usually detected is the surface usually white with blister and some reddish spotting because of the vascularization variation (Anggowarsito, 2014). It might leave hypertrophic scar (Hidayat, 2013).

Third degree burns

Third degree burns affect the entire thickness of the skin and deeper layers such as hypodermis, fat, muscles, tendons, and even bones. The microscopic features of this category are damaged skin organs, no epithelial elements remain, and blood vessels thrombosis. Loss of sensation and the white, gray, or black color of the burn skin are the physical characteristics of it. Healing process occur for a long time because there is no spontaneous epithelialization (Wardhana, 2014; Hidayat, 2013).

2.2.3 Burn wound healing process

Healing process of a burn wound in general has the same phases like other wound healing, start with inflammation phase, then proliferation phase, and end with remodeling phase. Although there are some difference such as the degree of systemic inflammation and the the healing time, burn healing time is various because it depends on depth of the burns (Wardhana, 2014; Tiwari, 2012).

Inflammatory phase

Inflammation happened as soon as tissue gets injured to maintain hemostasis and this phase usually occur for two until three days (Wardhana, 2014). The inflammatory phase brings neutrophils and monocytes to the site of injury via vasodilation and fluid extravasation, that later will initiate an immune response and will also reduce necrotic tissue and activate signals for wound repair (Tiwari, 2012; Reinke and Sorg, 2012). This phase usually occurs for two until three days (Wardhana, 2014).

Proliferation phase

Proliferation phase is the re-revitalization process of the wound that happen after the inflammatory phase. This phase is characterized by fibroblast migration, deposition of the ECM, angiogenesis and the formation of tissue (Enoch and Leaper, 2005). Fibroblasts are the dominant cell that found in this phase as it is responsible to synthesis collagen (Grabb and Smith, 2013; Sudjarmiko, 2011). Proliferation phase usually occur from the third day until the third week of wound healing process (Wardhana, 2014).

Remodeling phase

Remodeling is the final and the longest phase of wound healing process. This phase usually lasts for six months until one year. A dynamic process occurs in the form of collagen remodeling, wound injury and scar maturation. The synthesis and degradation activity of collagen is in balance, also decreases of vascularization. At the end of this phase, scar tissue (cicatrix) which is pale, thin and weak and easily moved from the base produced and it has 80% normal skin strength (Gurtner and Wong, 2014).

2.3 Fibroblast

Fibroblasts are large, flat, stellate or spindle-shaped connective tissue cells with elongated nucleus and one or two nucleolus with chromatin that found in virtually all animal organs (**Figure 2.3**) (Chen *et al.*, 2012). They are mesenchymal origin and the exact form of fibroblasts varies depend on their site of action and function (Baum and Duffy, 2012). Fibroblasts are called permanent cells because they have ability to grow and regenerate for life. Fibroblast cells are also responsible for collagen and elastin synthesis and organization of extracellular matrix components (Porter, 2007).

Fibroblasts thereby define the appearance and strength of connective tissue and they play an important role in wound repair following tissue damage. When a tissue is injured, healing process happen in three phases, such as inflammation, proliferation, and remodeling. Fibroblast proliferation happen in proliferation

phase. Fibroblast proliferation is important to determine the final result of healing process. Fibroblast undergo mitosis (replication and division) and deposit new proteins, thereby aiding the healing process (Bainbrige, 2013).

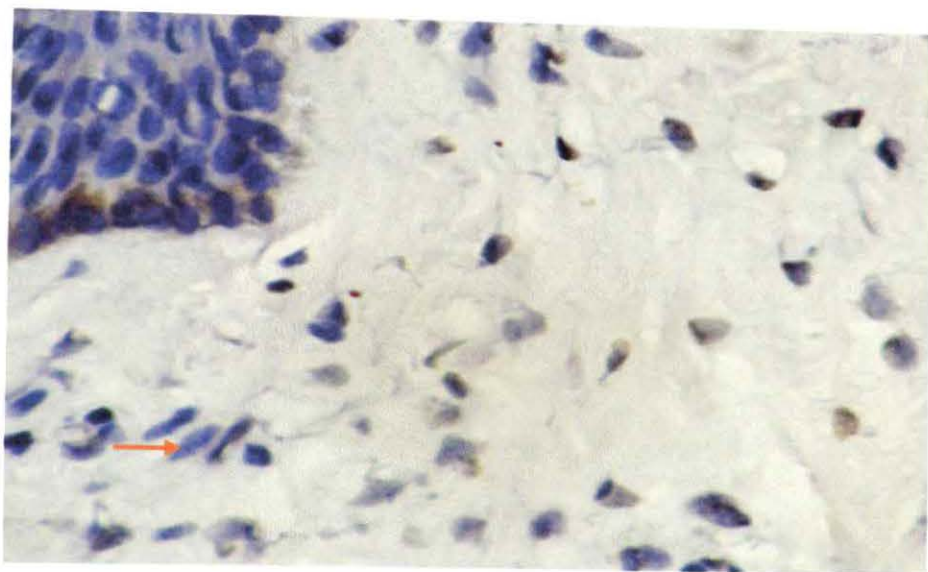


Figure 2.3 Fibroblast (orange arrow) in normal skin (Chen *et al.*, 2012)

2.3.1 Fibroblast role in wound healing process

Fibroblast proliferation is promoted by Interleukin-1b (IL-1b), Fibroblast Growth Factor (FGF), and Platelet-Derived Growth Factor (PDGF). Fibroblast migration to the needed area is promoted by Transforming Growth Factor-Beta (TGF- β) (Sumbayak, 2015). FGFs have been utilized for the regeneration of damaged tissues, including skin, blood vessel, muscle, adipose, tendon/ligament, cartilage, bone, tooth, and nerve (Yun *et al.*, 2010; Widyarini, 2016).

After fibroblast proliferation, there will be an increase in the number of cells and other healing factors. Proliferation of fibroblasts affects the final result of wound healing (Sumbayak, 2015). Fibroblasts produce and organize the extracellular matrix (ECM) by laying down a collagen rich matrix, communicating

with each other and other cell types (Sorrell and Caplan, 2004). Fibroblast also producing collagen, to unite the wound area and to affect the re-revitalization process at wound closure, and form new connective tissue and provide strength to generate a good healing process. Increasing number of fibroblast will increase the amount of collagen fiber that will speed up the wound healing process (Sumbayak, 2015).

Fibroblast will synthesis new collagen fiber from the third day after inflammatory phase. Collagen synthesis process will happen until several weeks depend on the size of the wound and this process is known to affect the strength in the wound area. After that, there will be a contraction between myofibroblast and epithelialization that makes wound area become narrower until wound healed. Result of wound healing process can be seen in formation of cicatrix (Sumbayak, 2015; Hidayat, 2013).

2.4 Madeira Vine

2.4.1 Madeira vine classification

Kingdom	: Plantae (plants)
Subkingdom	: Tracheobionta (vascular plant)
Super Division	: Spermatophyta (yielding seeds)
Division	: Magnoliophyta (flowering plants)
Class	: Magnoliopsida (double-dikotil)
Subclass	: Hamamelidae
Order	: Caryophyllales
Familia	: Basellaceae

Genus : *Anredera*
Species : *Anredera cordifolia* (Ten.) Steenis (**Figure 2.4**) (Susetya, 2014; Suhilman, 2016)



Figure 2.4. Binahong leaf (Suhilman, 2016)

2.4.2 Morphology

Madeira vine (*Anredera cordifolia* (Tenore) Steenis) also known as Binahong in Indonesia or 'lamb's tail vine' or 'mignonette vine', is a semi-succulent climbing vine that belongs to the family Basellaceae. Madeira vine is originated from Brazil and spread to Southeast Asia, European countries, and tropical America (Wagner *et.al*, 1999). This plants grows well in tropic and sub-tropic weather (Pink, 2004).

Madeira vine consists of leaves, stems, roots, and flowers. This plant vine up to 6 meters tall and it has fleshy, waxy green, heart-shaped leaves which usually 5-10 cm long and 3-7 cm wide. The stems are slender, soft, often reddish, twisted, and hairless. The root type is tuberous and has soft flesh. The flowers have five strands of creamy white crown that not attached and length for 0.5-1 cm. The flowers are approximately 10 cm long and are made up of numerous small flowers along a

drooping, central stem (**Figure 2.5**). Flowers of Madeira vine bloom from December to April and have a great fragrant (Susetya, 2014; DAF, 2016).

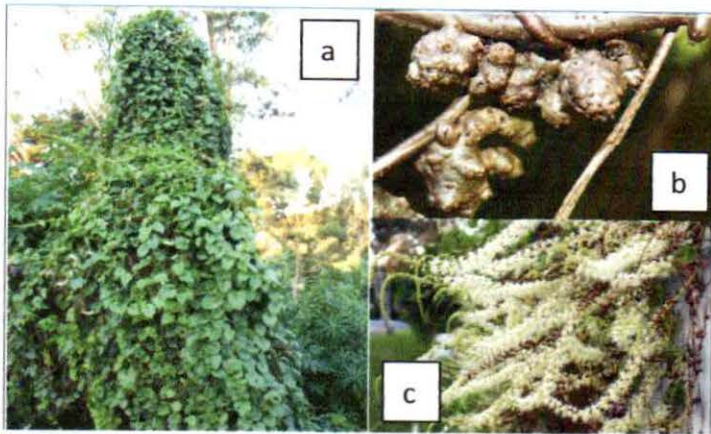


Figure 2.5 Madeira vine plant: (a) Madeira vine spreading (b) roots (c) flowers. (DAF, 2016)

2.4.3 Substances of Madeira vine

Almost all parts of this plant such as its bulbs, stems and leaves can be used in herbal therapy (Yuswantina, 2009; Ferri, 2009). One of the common use of Madeira vine as an herbal therapy is to help speed up the healing process of wounds because it contains active compounds such as fenol, terpenoid, steroid, saponins, flavonoids, tannins, and alkaloids (**Table 2.2**) (Astuti *et al.*, 2011; Hanafiah *et al.*, 2016). Saponin, terpenoid, and steroid substances are only found on the stems and leaves of the plant, however alkaloid compounds can be found on the leaves, tubers, and stems of the plant (Astuti *et al.*, 2011).

That makes the leaf of this plant is the most common part that been used in herbal therapy and scientific experiment. Research experiment that conducted by Hanafiah *et al.* (2016) shows that Madeira vine leaf extract has chemical compounds, such as saponins, tannins, alkaloids, triterpenoid, flavonoid, phenolics,

steroids, and glycosides. Madeira vine leaves also have such benefits as anti-inflammation, antioxidant, antibacterial, and analgesics (Suratman *et al*, 1996; Sasidharan et al., 2010; Yuziani *et al.*, 2014).

Table 2.2 Analysis of phytochemical compounds in Madeira vine plants

No	Compound	Leaf	Trunk	Flower	Root	Observation
1	Fenol	+	+	+	+	Reddish sediment
2	Flavonoid	+	+	+	+	Pink solution - red
3	Saponim	+	+	+	+	Permanent foam
4	Terpenoid	+	+	+	+	Reddish brown color, between surfaces
5	Steroid	+	+	+	+	Reddish brown / green - a thick bluish
6.	Alkaloid	+	+	-	+	Turbidity and sediment

Source: Astuti *et al.*, 2011

Madeira vine leaf also contain active compounds called oleanic acid, works as an anti inflammatory, and vitamin C that works on improving fibroblasts and neutrophils (Hapsari *et al.*, 2017). Madeira vine that been extracted with maceration process by using petroleum ether, ethyl acetate and ethanol solvent content alkaloid, saponin and flavonoid (Rochani, 2009). Research experiment conducted by Yuniarti and Lukiswanto (2017) shows the ideal extract percentage of using Madeira vine leaves extract to help speed up the wound healing process of burns is 5%.

2.4.4 Substances activity of Madeira vine leaf

a. Flavonoid

Flavonoids are one of the largest groups of natural phenol compounds in plants and composed of 15 carbon atoms. The core of it composed of a C6-C3-C6 configuration (Sriningsih *et al.*, 2008). One of the active substances that help to speed up the healing process of wound is Flavonoid (Astuti *et al.*, 2011). Flavonoid known to have the potential as antioxidant, antibacterial, anti-aging, anti-leukemic, and vasodilator activity (Hanafiah *et al.*, 2016).

It also has anti inflammation activity like saponin that shorter the inflammatory reaction through a few mechanisms, such as binding free radicals directly, through the inhibition of nitric oxide induction, decrease in leukocyte adhesion in the blood vessels, prevention of neutrophil degranulation, and interaction with other enzyme systems, and inhibited the TGF- β proliferative ability. TGF- β plays a role in migratory stimuli and fibroblast proliferation that will synthesize collagen which will help to speed up the wound healing process (Pakyari *et al.*, 2013; Nijveldt *et al.*, 2001).

Flavonol is the type of flavonoid that obtained from the isolation and identification of fresh powder and dry powder of ethanol extract from the leaves. The total flavonoid in fresh samples of Madeira vine leaf extract from dry and fresh samples were 7.81 mg / kg and 11.23 mg / kg. The content of flavonoids in fresh samples is greater, because in the process of preparation of fresh samples are not warmed up. The heating process can make the measure of flavonoid compounds

reduced. This heating process can lead to a decrease in total flavonoid levels by 15-78% (Selawa *et al.*, 2013).

b. Saponin

Saponin is one class of compounds glycosides, steroids, alkaloid steroid, and triterpenoid (Konoshima *et al.*, 1995; Wallace *et al.*, 2002). Saponin have known to have role as antibacterial, antifungi, antiinflammatory, antioxidants and analgesic agents (Harborne, 1973; Singh, 2006; Blumert and Liu, 2003; Olezek *et al.*, 1990). It also plays a role in influencing the activity and synthesis of TGF- β 1 and modification of the receptors TGF- β 1 and TGF- β 2 in fibroblasts which will stimulate the synthesis of fibronectin to form collagen matrix (Kaur *et al.*, 2014).

Saponin is able to speed up the wound healing process by influencing fibroblasts proliferation to stimulate the formation of collagen (Suratman *et al.*, 1996). In low concentration saponins can be hemolysis of red blood cell (Harborne, 1973). Saponin also have the ability to act as a cleanser and possesses antiseptic properties which serve to kill and prevent the growth of microorganisms that arise in the wound, so that the wound does not experience severe infections (Paju *et al.*, 2013).

The leaves of Madeira vine contain saponins compound, such as glycosides, terpenoid, steroid, and alkaloid. The content of saponin compound quantitatively in binahong plant showed saponin percentage of every 20 mg of dried samples contained in leaves and plant roots with the amount of (28.14 ± 0.22) for leaf, trunk (3.65 ± 0.11) and roots (43.15 ± 0.10) (Astuti, 2013). The extract of saponins

compounds will give better results when using a solvent such as ethanol (Harborne, 1973).

c. Tannin

Tannins are a class of astringent that can cause skin pores to shrink, polyphenols that either bind and precipitate or shrink proteins (Ashok and Upadhyaya, 2012). Tannins characterized as light yellow or light brown or white amorphous powders or flakes, nearly colorless, loose masses, with a characteristic strange smell. Tannins are found almost in all plants in all climates all over the world (Falbe and Regitz, 1995; Ashok and Upadhyaya, 2012).

Tannins have role as antioxidant and antimicrobial that preventing and protecting wound area from being damaged by free radicals and inhibits the growth of pathogenic bacteria in the wound (Ashok and Upadhyaya, 2012). Tannin also have role in fibroblasts migration and proliferation to speed up the wound healing process (Ashok and Upadhyaya, 2012; Kimura *et al.*, 2006).

2.5 Turmeric

2.5.1 Turmeric Classification

Kingdom	:	Plantae
Division	:	Spermatophyta
Class	:	Monocotyledoneae
Order	:	Zingiberales
Family	:	Zingiberaceae
Genus	:	Curcuma
Species	:	<i>Curcuma longa</i> Linn (Figure 2.6) (Sabale <i>et al.</i> , 2013)

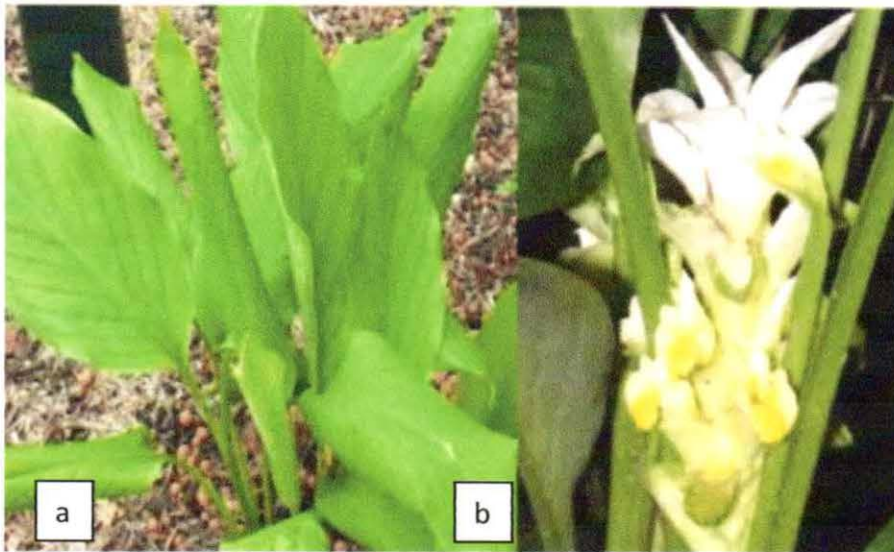


Figure 2.6 Turmeric plant: (a) leaves, (b) flowers (Sabale *et al.*, 2013).

2.5.2 Morphology

Turmeric (*Curcuma longa*) is also known as Kunyit in Indonesia. It is an herb that originated from Southeast Asia, distributed throughout tropical and subtropical area, and widely cultivated in South Asia especially in India, China, Taiwan, Indonesia, and the Philippines (Sumiati, 2004). It can grow up to one meters high with a short stem. It has single, oval shape, pointed leaves with pale green color, funnel-shaped about 10-15 long with a crown about 1.5 to 3 cm wide white-yellowish flowers, and brown skin of rhizome with dark orange flesh (**Figure 2.7**) (Sumiati, 2004; Nasri *et al.*, 2014; Joseph, 2019).

Turmeric has been used as a spice, in medicine, and for coloring and flavoring food. (Jovičić *et al.*, 2017). It has been authenticated as anticancer, anti-diabetic, antioxidant, anti-inflammatory, antimicrobial, anti-venom, etc (Nasri *et al.*, 2014). The part of turmeric that used in medicine and food is the rhizome.



Figure 2.7 Turmeric Rhizome (Joseph, 2019)

2.5.3 Substances of Turmeric rhizome

Turmeric is very rich in phenolics, whose structures have been identified as curcuminoids, which are chemically related to its principal ingredient, curcumin (Ammon and Wahl, 1991; Eigner and Scholz, 1999). Main compound of turmeric rhizome is volatile oil and curcuminoid. Curcuminoid is a crystal-shaped prism or short stem, emulsion or water-insoluble but easily soluble in acetone, ethanol, methanol, bensen and chloroform. In addition to curcuminoids and essential oils, turmeric rhizome also contains other compounds such as starch, fat, protein, camphor, resin, resin, gum, calcium, phosphorus and iron (Hartati, 2013)

Curcuminoid consist of curcumin and its derivates are demotoxicurcumin and bisdemotoxicurcumin. Cur (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), also called diferuloylmethane, is the natural yellow pigment in turmeric, it is isolated from the rhizomes of the plant (Hartati, 2013; Hewlings and Kalman, 2017). In the crude extract of Turmeric rhizomes about 70–76% curcumin is present along with about 16% demethoxycurcumin and 8% bisdemethoxycurcumin (Kohli *et al.*, 2004).

2.5.4 Mechanism activity of Curcumin

Curcumin known to have roles as anti-inflammatory, anti-spasmodic, antimicrobial, anticancer, antioxidants, hepatoprotection and neuroprotection, etc. It is interesting that curcumin is lack of gastrointestinal side effects despite being an anti-inflammatory agent (Kohli *et al.*, 2004). Curcumin has also been found to enhance cutaneous wound healing through involvement in tissue remodeling, granulation tissue formation, and collagen deposition (**Table 2.3**) (Joe *et al.*, 2004; Akbik *et al.*, 2014).

Table 2.3 Summarizing the effects of curcumin topical treatment on different stages of wound healing

Wound Healing Stage	Effects of Curcumin Topical Treatment
Inflammation	• Inhibiting the activity of NF-β transcription factor, reducing the production of TNF- α and IL-1 cytokines, and thereby reducing inflammation • Increasing production of anti-oxidant enzymes (dose dependent)
Proliferation	• Enhancing fibroblast migration, granulation tissue formation, collagen deposition, and in general re-epithalization • Being apoptotic in the early phase of wound healing, thereby eliminating unwanted inflammatory cells from wound site
Remodeling	• Improving wound contraction by increasing the production of TGF- β and therefore increasing fibroblast proliferation

Source: Akbik *et al.*, 2014

Curcumin known to increase the migration of myofibroblasts, and macrophages that play an important role in wound healing process after burn injury (Mehbrani *et al.*, 2015) Curcumin may be useful in conditions of impaired wound healing, as it appears to stimulate the production of TGF- β 1 in the wound which play a role in fibroblast proliferation (Sidhu *et al.*, 1998; Mani *et al.*, 2002). Research conducted

by Kulac *et al.* shows that there was a rise in the expression of proliferating cell nuclear antigen in skin tissues of curcumin treated rat models suffering from burn lesions substantiating the beneficial effects of the topical application of curcumin in acceleration of wound healing (Kulac *et al.*, 2013). Curcumin was shown to be Research experiment conducted by Mehbrani *et al.* (2015) shows the ideal extract percentage of curcumin to be a suitable substitute in healing of burn wounds is 2%.

2.6 Gel Base

Gel is one of the topical formation that has high water content and can cause hydration of the stratum corneum that help facilitate the penetration of the drug through the skin. Gels are semisolid systems consisting of either suspension composed of small inorganic particles or large organic molecules interpenetrated by a liquid (USP, 2009). The composition of gel base will affect its physical properties (Mohamed, 2004).

Gel can be classified based on the solvent, such as hydrogels, organogels, and xerogels. Hydrogels are hydrophilic polymeric gels that can swell in water and carry huge amounts of water while maintain the structure of drug that carried by it (Anumolu, 2015). Hydrogel for dermatology has several properties such as greaseless, easy to spread, easy to remove and emollient (Mohamed, 2004). In case of burns, if cleaning water is not available for first aid, application of hydrogels onto the wound is the only way to cool the wound, in order to minimize the extent of damage and reduce pain. The high water content of hydrogels makes them particularly cooling and soothing on the wound area. Hydrogels are also non-adhesive, since cells do not readily attach to highly hydrophilic surfaces. This

implies that hydrogel dressings do not stick to the wound, highly facilitating the change of the dressing by causing less pain and discomfort to the patient. On the other hand, these hydrogels not only absorb exudates but also prevent from the loss of evaporative water and the wound dehydration, thus facilitating wound healing (Bhattarai *et al.*, 2010; Jayakumar *et al.*, 2011).

2.7 Silver Sulfadiazine

Silver Sulfadiazine (SSD) cream is the standard medicine in burns treatment, especially in second-degree and third-degree burns, that had been used clinically over three decades since Fox first synthesized from silver nitrate and sodium sulfadiazine with 1% concentration for each (Fox, 1983; Rahma, 2014).

However, the use of SSD has been associated with delayed healing of wounds in which the process of skin cell proliferation and collagen deposition. SSD also known to cause black marks and the silver element of SSD was shown to be highly toxic that can decrease the accuracy of wound healing scoring by forming slough layer. Slough layer is a layer or mass of dead tissue separated from surrounding living tissue (Saedinia, 2017).

The cytotoxic effect of SSD on the fibroblast is likely to inhibit the proper maturation of the collagen matrix (Cho Lee and Kyung Moon, 2003). The conventional anti-infective property of SSD can not provide the moisture to boost a rapid wound healing (Homann *et al.*, 2007). Despite all the disadvantages of SSD, it is still the first choice of medicine for burn wounds in general population (Sevgi, 2014).

2.8 White Rat

White rats are an omnivorous animal which can weight up to 500 g with 190-265 mm body length and 160-205 mm tail length (**Figure 2.8**) (Akbik, 2010). Sprague Dawley, Long Evans, and Wistar are three common strain of white rats that usually used as the laboratory experiment animal (Wandari, 2014). Male white rats were generally used for research experiments. This is because male rats have more stable reproductive hormones than the female. Female rats are easier to get stress because of their unstable reproductive hormones (Andini, 2016).

White rat's classification is:

Kingdom	: Animalia
Filum	: Chordata
Kelas	: Mamalia
Ordo	: Rodentia
Famili	: Muridae
Genus	: Rattus
Spesies	: <i>Rattus norvegicus</i> (Adiyati, 2011).



Figure 2.8 White rat (*Rattus norvegicus*) (Akbar, 2010)

CHAPTER 3

MATERIALS AND METHODS



CHAPTER 3 MATERIALS AND METHODS

3.1 Experimental Design

This research is going to use laboratory experiment type using random sample. This research will use the experiment group and the control group. The research is conducted to see the effect of Madeira Vine leaves extract (*Anredera cordifolia* (Ten.) steenis) combined with Turmeric rhizome extract (*Curcuma longa*) in gel form given tropically in deep second-degree burn wound of white rat (*Rattus norvegicus*), Wistar strain.

3.2 Sample and Sample Size

Sample that is going to be use in this research is the skin of white mice (*Rattus norvegicus*) which collected at the 15th day, after the burn injury. Sample size is 1 cm x 1 cm and will be put into 10% buffer formalin before being made into histological preparations. Rats that is going to be use are male and in healthy condition without any skin diseases.

3.3 Research Observed Variable

Independent Variable : Madeira vine leaves extract combined with 2%

Turmeric rhizome extract in gel form at three concentrations of 1,25%, 2,5%, and 5%.

Dependent variable : number of fibroblast that observed with the histological preparation of the skin burn in the 15th day.

Controlled Variables : rat (type, weight, age, sex, and food), the burn wounds degree, environment (cage), and treatment time range.

3.4 Operational Variable Definition

- a. The deep second-degree burn wounds in this research experiment are intentionally formed using a modified thermostat that has 1 cm diameter with temperature $85\pm 5^{\circ}\text{C}$ in 5 seconds (Abdeldjelil *et al.*, 2017). The deep second-degree burn wound is already include the reticular dermis and the healing process usually take 2 to 5 weeks with scar (Garcia-Espinoza *et al.*, 2017). The deep second-degree burn wound surface is usually white with blister and some reddish spotting because of the vascularization variation (Anggowarsito, 2014).
- b. This research will be using a topical treatment for the deep second-degree burn wounds. In this research there will be three treatment groups and one positive control group. Treatment groups will be treated with gel of Madeira Vine leaves extract combined with Turmeric rhizome that processed into three concentrations (1,25%; 2,5%; 5%). This preparation uses Nipagin as a gel base. The positive control group will be treated with the common burn wounds medicine which is Burnazin ointment that contain 1 % silver sulfadiazine.
- c. Result that will be observed is the number of fibroblast in histological preparation with Hematoxylin Eosin (HE) stain. Fibroblast will be seen as a Fibroblast calculation is carried out using modified method of Fuadi *et al.*, (2015).

- d. The experimental animals used in this study were male rats (*Rattus norvegicus*) aged 2-3 months with a weight of 200-300 grams obtained from breeders in Surabaya.

3.5 Research Location and Time

Herbal medicine preparation for this research take place in Pharmacology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga. The experiment for this research is conducted in Sample Animal Cage, Faculty of Veterinary Medicine, Universitas Airlangga. Histological preparation for this research is going to be conducted in Pathology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga. This research take time from October 2018 until January 2019.

3.6 Research Materials and Equipment

3.6.1 Research materials

Experimental Animal

Animals that being use in this research experiment are white rat (*Rattus norvegicus*), Wistar strain, with 200-300 gram weight and in healthy condition. According to WHO, the minimum number of sample is 5 in each group. This sample size determined by Federer fomula (Muntaha et al., 2015) :

$$(t - 1)(n - 1) \geq 15 \geq 15$$

Information :

t = number of treatment

n = number of repetition

$$\begin{aligned}
 \text{Calculation} &= (t - 1)(n - 1) \geq 15 \\
 &= (5 - 1)(n - 1) \geq 15 \\
 &= (n - 1) \geq 15/4 \\
 &= n \geq 3.75 + 1 \\
 &= n \geq 5
 \end{aligned}$$

The minimum number for each treatment groups is 5 samples. The total number of rats used in this research experiment is 25.

Other materials beside the experimental animals are the ingredients for the maintenance the rats are feed, water, and sawdust, silver sulfadiazine as a standart treatment, ketamine and xylazine as anesthetics and euthanasia, and the ingredients for making extract and gel were Madeira Vine leaves, Curcumin, 96% ethanol, Carboxy Methyl Cellulose (CMC), glycerin, nipagin, propylene glycol and aquades. This research materials for making histopathological preparations used HE staining, alcohol (70%, 80%, 90%, and 96%), xylol (I, II, and III), paraffin (I and II), and 10% formalin.

3.6.2 Research equipment

The equipments for experiment animals are cages, wires, bulkheads, water container and feed containers. The equipments for forming the burn wound are modified thermostats, hair shavers, syringe, and thick gloves to hold the heater. The equipments for making extracts and gel are ovens, water baths, evaporators, filter paper, scales, cups, hot plates, stirers and gel containers. The equipments for the topical treatment are sterile cotton buds, sterile cotton, NaCl and camera for documentation. The equipments for sampling the rat skin are surgical instruments

such as scapel, blades, syringe, and tweezers. Another equipment is a container for organs that will be included with 10% formalin. For histopathology making, several tools are needed, including tools for dehydration, microtome, water bath, cover glass, object glass, and so on. Observation of preparations was carried out under a microscope and documented using optilab.

3.7 Research Procedure

3.7.1 Madeira vine leaf extract and Turmeric rhizome extract procedure

Madeira Vine leaf and Turmeric rhizome that had been obtained from Dukuh Kupang village, Dukuh Pakis Sub-district, Surabaya City were washed, drained, cut into small pieces and then dried by aerating and carried in an oven with a temperature of 40°C until dry. The dried Madeira Vine leaves and Turmeric rhizome are grind until it become powder.

Powders of Madeira Vine leaf and Turmeric rhizome are soaked in ethanol 96% solvent with 1:10 ratio and adjusted in a measuring cup with occasional stirring for five days, this is according to Indonesian Pharmacopoeia. After five days, separate the first debris and the first filtrate using filter paper. Next, soaked the first debris again in ethanol 96% for two days with occasional stirring. After two days, separate the second debris and the second filtrate using filtrate paper.

After having the first filtrate and the second filtrate, mix it together and filter again to make sure there are no debris left. The filtrate was then evaporated using a vacuum evaporator with a temperature of 60°C so that the extract was almost viscous and continue using a water bath with a temperature of 60°C until viscous extract was obtained (Paramita, 2016).

3.7.2 Madeira vine leaf extract combined with Turmeric rhizome extract in gel form procedure

After the viscous extract had been achieved, the gel base materials were prepared in advanced and weighed according to the formulation. The formulation was made into three different concentration of Madeira Vine leaves extract such as 1.25%, 2.5%, and 5% combined with 2% Turmeric rhizome extract in each. Each formulation was made into 25g gel. (Appendix 1).

3.7.3 Forming II B burn wound procedure

The rats were anesthetized with the combined of Ketamine and Xylazine (100 mg/kg body weight [BW] and 5 mg/kg BW) with intramuscular injection (Flecknell and Thomas, 2015). Gluteal dexter of rats was shaved and smeared with Alcohol 70%. Then, the modified thermostat (Appendix 3) was heated first until it reached 85°C for around 15 minutes. After that, applied the modified thermostat to the shaved area for 5 seconds with vertical position to form the deep second-degree burn wound.

3.7.4 Burn wound treatment procedure

A total of 25 males, Wistar rats were put randomly in five groups, each groups receiving different treatments. The treatments are:

C- : Rats with normal healthy skin

C+ : Rats with skin burns, treated with Silver Sulfadiazine (SSD) 1 %

- P1 : Rats with skin burns, treated with gel of Madeira Vine leaf extract combined with 2% Turmeric rhizome extract (MVLTR) with 1.25% concentration
- P2 : Rats with skin burns, treated with gel of Madeira Vine leaf extract combined with 2% Turmeric rhizome extract (MVLTR) with 2.5% concentration
- P3 : Rats with skin burns, treated with gel of Madeira Vine leaf extract combined with 2% Turmeric rhizome extract (MVLTR) with 5% concentration

The treatment was carried out topically by smearing the gel of MVLTR and SSD using sterile cotton buds. The treatment was conducted twice daily for 14 days, starting from the first day of burn wound was made.

3.7.5 Rat euthanasia procedure

After 14 days of treatment, experiment animals were sacrificed with an overdose anesthetic on the next day, Ketamine 90 mg/kg combined with Xylazine 10 mg/kg intraperitoneally (Kulac *et al.*, 2012) were used as the anesthetic as they known to have no vital functions depressed (Zandieh *et al.*, 2003), and the burn areas of the skin were removed for histological studies. Sample size is 1 cm x 1 cm and will be put into 10% formalin before being made into histological preparations.

3.7.6 Skin histological preparation

Skin histological preparation was made using paraffin method and the stain that used in this process is Hematoxylin-Eosin (HE). The paraffin method is divided into eight processes such as, specimen collection, fixation, dehydration, clearing, blocking, sectioning, staining, and mounting (Hestianah *et al.*, 2016). The detail of skin histological preparation steps can be seen in Appendix 2.

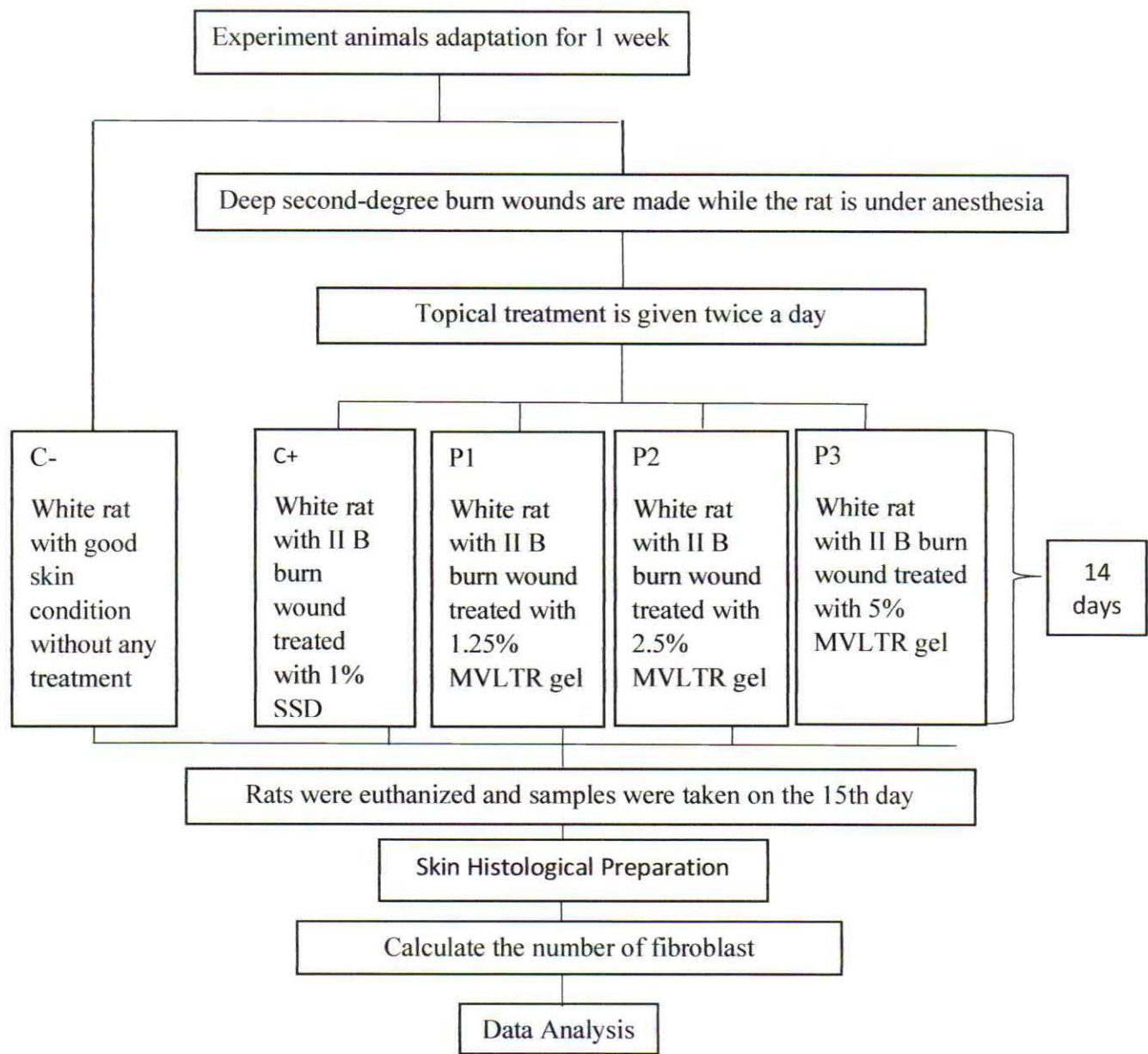
3.7.7 Number of fibroblast calculation

The samples were observed with Optilab and Nikon 600L microscope. The number of fibroblast was calculated in histopathic form with Image Raster application. The histology preparations were stained with Hematoxylin-Eosin (HE) stain and the observations of the fibroblast were made using the modified Fuadi *et al.* (2015) method. The number of fibroblast was calculated at 400x magnification in five field of view.

3.8 Data Analysis

The average data of fibroblast number for each treatment was analyzed using the Statistic Product Service Solution (SPSS) 20.0 program with One Way Analysis of Varian (ANOVA) and the Duncan method to see the significant value between treatments with the significant differences value $p < 0.05$ (Fuadi *et al.*, 2015).

3.9 Research Flowchart



CHAPTER 4

RESEARCH RESULT



CHAPTER 4 RESEARCH RESULT

This research observed the effect of giving Binahong leaf extract with three different concentration combined with 2% concentration of Turmeric rhizome extract in gel form for the healing process of burn wound on the white rat skin seen from the number of fibroblast. The ointment was given twice a day for 14 days and on the 15th day the skin organ was processed into histological preparation. There were five treatments in this research, C-, C+, T1, T2, and T3. C- is the healthy rat skin as the control negative in this research, C+ is the rat skin with burn wound treated with SSD as the control comparison in this research. T1, T2, and T3 are the rat skin with burn wound treated with Binahong leaf extract combined with 2% of Turmeric rhizome extract gel and distinguished by Binahong leaf extract concentration which are 1.25%, 2.5%, and 5%. Each treatments group consist of five repetitions.

The skin histological slide was stained with HE. The skin histological slide was seen with microscope at 100x magnification at first to confirm the healing point area, then five fields of view of the skin histological slide were seen using microscope at 400x magnification and an Optilab. Number of fibroblast was counted from five fields of view per each treatments group per each repetitions using Image Raster application and the data were showed in average number of fibroblast each treatments group (**Appendix 4**).

The data is then entered into SPSS program to be analyzed. First, data normality is checked and based on Kolmogorov-Smirnov the results obtained are $p > 0.05$, which means that the data is normally distributed. Next, difference between

treatments group is checked with One Way ANOVA and based on it the results obtained are $p<0.05$, which means the difference between treatments group are real. Last, the processing is continued using the Duncan method to see which treatments group has the highest and lowest data. The detailed of data analysis process can be seen in **Appendix 5**. The mean and standard deviation of Fibroblast from C+, C-, T1, T2, and T3 can be seen in **Table 4.1** and **Figure 4.1**.

Table 4.1. Fibroblast on IIB Burn Wound of White Rat Treated with Madeira Vine Leaf Extract Combined with Turmeric Rhizome Extract Gel

Treatment	Fibroblast (Mean ± Standard Deviation)
C-	11.44 ^a ± 1.84
C+	35.72 ^c ± 2.81
T1	27.56 ^b ± 3.73
T2	41.76 ^{cd} ± 6.18
T3	47.40 ^d ± 7.42

Note: Different superscript shows significant difference between treatments ($p<0.05$)

The result of the examination found that C- is significantly different with C+, T1, T2, and T3 ($p<0.05$). C+ is significantly different with T1, T3 ($p<0.05$) and not significantly different with T2 ($p>0.05$). T2 is not significantly different with C+ and T3 ($p>0.05$).

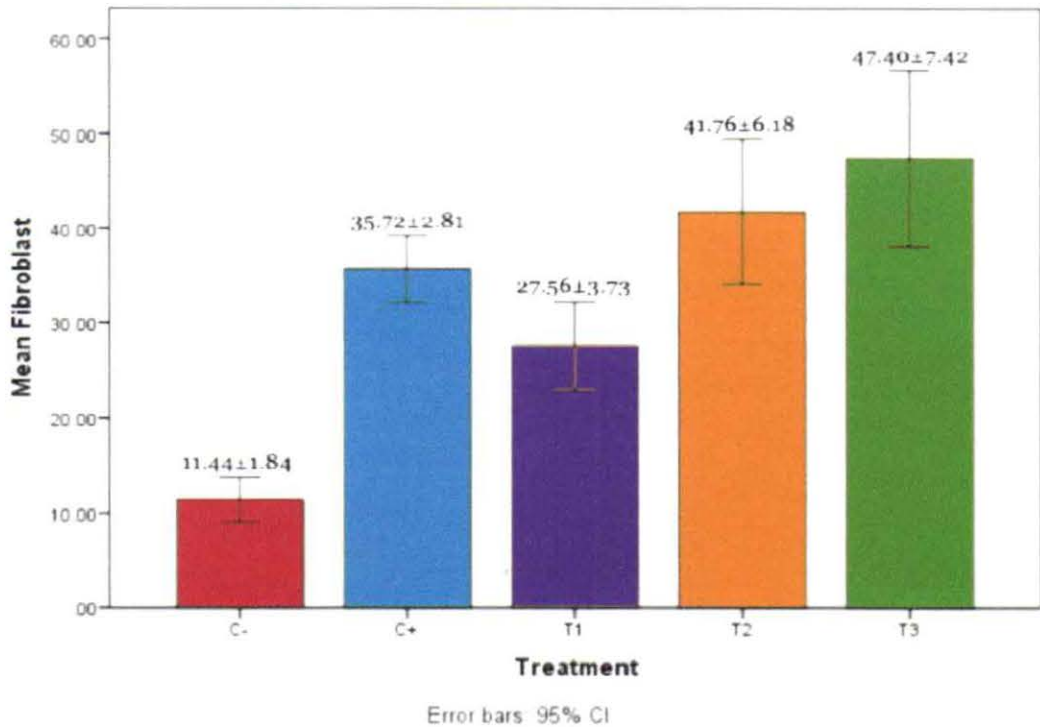


Figure 4.1 Average Number of Fibroblast and Standard Deviation per Treatment

In the graph, it can be seen that T3 has the highest average number of fibroblasts, which is 47.40 ± 7.42 . The second place is T2 with 41.76 ± 6.18 . The third place is C+ with 35.72 ± 2.81 . The fourth place is T1 with 27.56 ± 3.73 . Furthermore, the lowest average number of fibroblast is C- with 11.44 ± 1.84 .

From **Figure 4.2**, it can be seen that re-revitalization has been formed in all treatments. From the color and space between collagen in C- it can be seen that the collagen is mature, fibroblast is hard to find and the number of fibroblast is the lowest among five treatments, then in C+ the collagen is immature and the number of fibroblast is at the third place among other treatments. In T1 the collagen is also immature and the number of fibroblast is at the fourth place among other treatments.

Collagen of T2 and T3 are starting to mature and fibroblasts are easy to find. However, T2 has lower number of fibroblast than T3 and it makes T3 as the treatment group with the highest number of fibroblast.

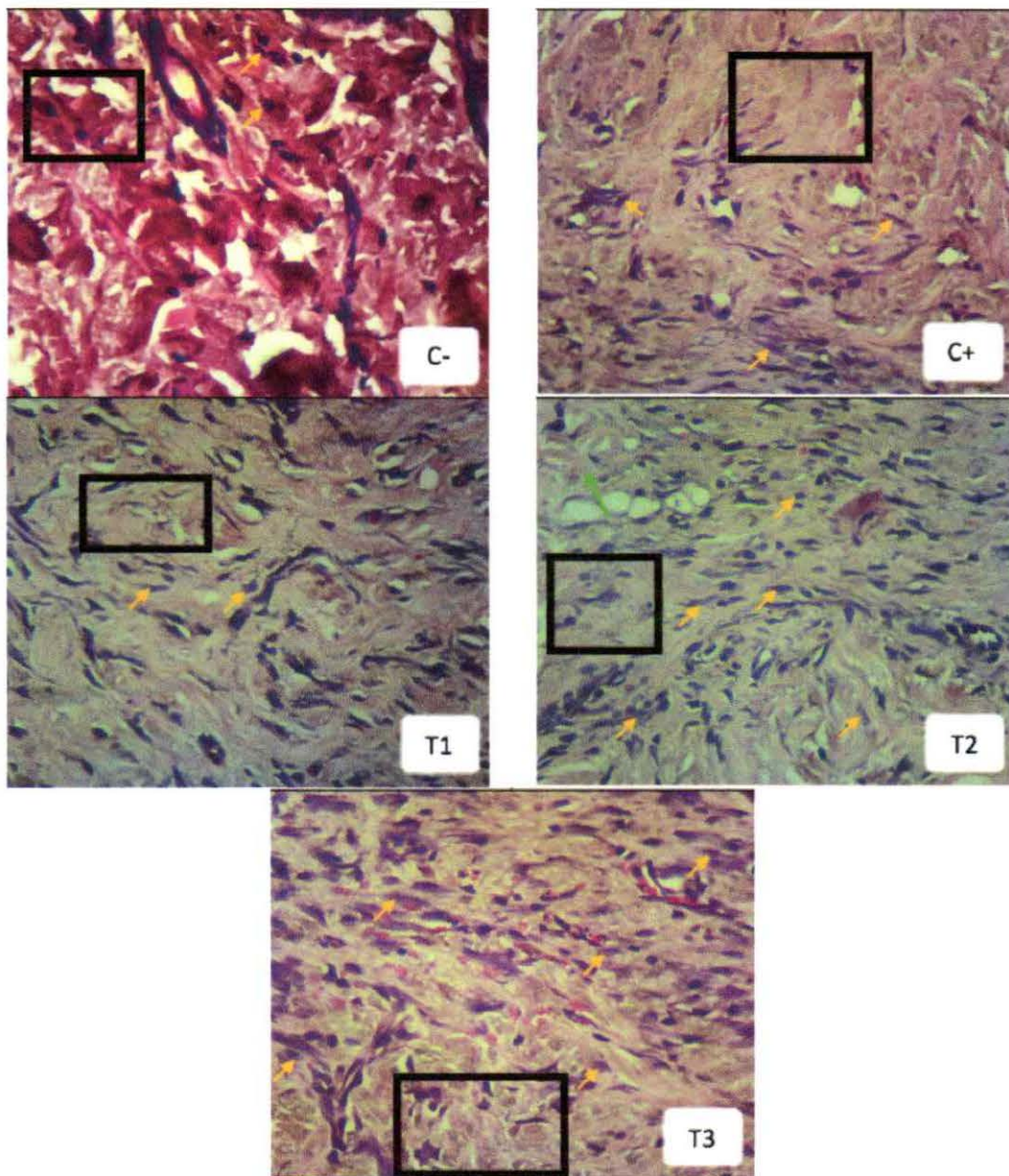


Figure 4.2. Histological appearance of rat skin in each treatment (HE, 400x); (→ show fibroblast, black rectangle show collagen); (C-) Normal healthy skin rat and has the lowest number of fibroblast; (C+) Skin with IIB burn wound treated with 1% SSD; (T1) Skin with IIB burn wound treated with 1.25% MVLTR gel; (T2) Skin with IIB burn wound treated with 2.5% MVLTR gel; (T3) Skin with IIB burn wound treated with 5% MVLTR gel and has the highest number of fibroblast.

CHAPTER 5

DISCUSSION



CHAPTER 5 DISCUSSION

In this study, the result of Binahong leaf extract combined with 2% Turmeric rhizome extract gel treatment on the number of fibroblast in II B burn wound was increasing. Based on the data the highest number of fibroblast was on T3 or 5% concentration of Madeira vine leaf extract combined with 2% Turmeric rhizome extract (MVLTR). Several studies have highlighted the wound healing effect of some treatment through stimulating fibroblasts and keratinocytes (Tsala *et al.*, 2014).

From the data it can be known, C- (the normal healthy skin) showed the lowest number of fibroblast. This is because in healthy skin, fibroblast is not really needed because the collagen is already mature, strong, and there is no damaged that stimulate the activation of fibroblast. T1 (1.25% MVLTR gel) was showed the second low number of fibroblast. This might cause by the amount of water in this ointment is too much which make the wound's humidity too high and could not heal properly (Kurahashi and Fujii, 2015). Humidity is one of the conditions affected wound healing process (Martins *et al.*, 2015).

C+ (SSD ointment) was showed a moderate value of fibroblast. SSD has been used as the standard treatment for burns and well known as the useful antibacterial

agent. SSD is known to cause black marks and the silver element is known to be highly toxic that could decrease the wound healing process (Saedinia, 2017), however the toxicity of silver has not been an issue in burn care that has received much attention (Poon and Burd, 2004). The result of this research has shown that C+ is having a better effect of wound healing process than T1 gel seen from the average number of fibroblasts found.

T2 (2.5% MVLTR) and T3 (5% MVLTR) was not significantly different from the data. Both of them showed a good sign of healing based on the fibroblast number and the preview of some collagen that started to mature (**Figure 4.2**). Based on the data, average number of fibroblast in T3 is higher than T2 and it is the highest of all treatments. This research was conducted for 14 days treatment and based on the observation on the slide of the skin, it can be seen that this wound is in the proliferative phase.

There are three phases in wound healing process which overlap with each other but also well-defined: inflammatory, proliferative, and remodeling (Enoch et al., 2006; Wardhana, 2014). The inflammatory phase usually occurs up for three days but burn wound may suffer from the effect of acute influx of inflammatory mediators and growth factors for a prolonged time. The burn wound management would involve reducing the extent of inflammatory response to acute injury and

regulating fibroblast and myofibroblast activity. The activity would reduce contracture without affecting wound healing and strength of wound repair (Evers *et al.*, 2010).

The second phase is proliferative phase which occurs from the third day until the third week. The final phase of wound healing process is remodeling phase which might last from six months up to one year. (Warhana, 2014).

Proliferation phase is characterized by fibroblast migration, angiogenesis, synthesis and deposition of fragments of ECM proteins such as collagen tissue and granulation tissue (Enoch *et al.*, 2006). Fibroblasts produce the new ECM to support cell ingrowth using collagen as the building blocks and thus makes it as the dominant cells of this phase.

According to the study from Harris *et al.* (1981), the fibroblast traction is the cause for apparent contraction of collagen networks around deep wounds, burns, and surgically implanted prostheses. Concentration and stabilization of collagen fibers that are increased also helps to improve the tensile strength (Nayeem and Karvekar, 2011). Tensile strength will make the wound is not easily torn apart again. The tensile strength of the skin caused by a type I collagen secreted by fibroblasts (Gurtner and Wong, 2007).

Remodeling phase involves collagen remodeling and scar tissue formation. Proliferative to remodeling phase are overlapping and this could make a big number of fibroblasts and some parts with almost-mature collagen seen in one field of view.

Madeira vine and Turmeric has been known to be use as traditional medicine to treat different kind of disease, in this research it is known that both of them also have a great effect of wound healing process especially on burn wounds. This caused by the substances in them. Madeira vine leaf is known to have high compound of flavonoid (Harborne, 1973) that work as antioxidant and antibacterial (Hanafiah *et al.*, 2016), saponin, tannin, and oleanolic acid. As flavonoid has direct roles as antibiotic function have broad spectrum target (Ferri, 2009).

Saponin works as anti-inflammatory and antimicrobial, such as anti-bacterial and anti-viral (Harborne, 1973). Saponin is able to speed up the wound healing process by influencing fibroblast proliferation to stimulate the formation of collagen (Suratman *et al.*, 1996). Tannin works as antioxidant and antimicrobial, also functions as an astringent that can cause skin pores to shrink and stop exudates and mild bleeding, causing wound to close and preventing bleeding on the wound (Ashok and Upadhyaya, 2012). The leaves also have oleanolic acid content which has anti-inflammatory properties that could reduces pain in burn (Hammond, 2006).

In the other hands, Turmeric rhizomes has curcumin as its principal ingredient which known to have roles as anti-inflammatory, antispasmodic, antimicrobial, anticancer, antioxidants, etc (Kohli *et al.*, 2004). Curcumin also known to stimulate the production of TGF- β 1 that works in fibroblast proliferation in wound healing process (Sidhu *et al.*, 1998; Mani *et al.*, 2002).

The benefits of the MVLTR gel was not only produce good conditions for wound healing, but there is also a possibility to accelerate the process of wound healing. Based on this study, it is known that T3 is the most effective treatment and optimal concentration. This study observes wound healing process on one of its factor which is fibroblast. The results of this study also show that MVLTR gel could have a potential to substitute SSD treatment as a treatment for burns because the MVLTR gel has better results, especially in the deep second degree burn.

CHAPTER 6

CONCLUSION



CHAPTER 6 CONCLUSION

6.1 Conclusion

Based on this research, can be concluded that Binahong leaf extract combined with 2% Turmeric rhizomes extract gel as topical ointment for deep second degree skin burn in rat can increase the number of fibroblast with the highest result at 5% concentration.

6.2 Suggestion

The suggestion that can be given according to this research is:

Further observations through other concentration of Binahong leaf extract and Turmeric rhizomes extract gel, day of observation, degree of burn, and variables of healing result that necessary in order to understand the complete effect of Binahong leaf extract combined with Turmeric rhizomes extract gel as topical ointment in burn wound.

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APPENDICES



APPENDIX

Appendix 1 Ointment Dosage Calculation

Gel combination of MVLTR formula

Preparation of gel combination used in this study using concentration of binahong leaf extract 1.25%, 2.5% and 5% and concentration of turmeric rhizome 2% made as much as 25 g.

- a. Gel combination of binahong leaf extract 5% and turmeric rhizome extract 2%

R /	Binahong leaf extract	1.25g
	Turmeric rhizome extract	0.5g
	CMC Na	5%
	Glycerin	10%
	Propilen Glikol	5%
	Nipagin	0.2%
	Aqua ad	25 g
	m.f gel	

- b. Gel combination of binahong leaf extract 2.5% and turmeric rhizome extract 2%

R /	Binahong leaf extract	0.625g
	Turmeric rhizome extract	0.5g
	CMC Na	5%
	Glycerin	10%
	Propilen Glikol	5%
	Nipagin	0.2%
	Aqua ad	25 g
	m.f gel	

- c. Gel combination of binahong leaf extract 1.25% and turmeric rhizome extract 2%

R /	Binahong leaf extract	0.3125 g
	Turmeric rhizome extract	0.5 g
	CMC Na	5%
	Glycerin	10%
	Propilen Glikol	5%
	Nipagin	0.2%
	Aqua ad	25 g
	m.f gel	

Appendix 2 Procedure of Histological Specimen Preparation

1. Organ Cutting

The first thing to do after the experimental animals euthanized is tissue immersion in preservative agent. Preservatives which usually used are Neutral Buffered Formalin solution (BNF) 10% with a pH range between 6.5 - 7.5. The ideal pH is 7.0. Comparison between the organ and the solution is 1: 10 so that the fixation of tissue with the solution is complete, while the length of fixation of at least 2 days.

2. Dehydration and Clearing

Drained the organ using filter then cut it using a scalpel blade with a thickness of 0.3 - 0.5 mm and arranged into a tissue cassette, then a tissue cassette is inserted into a special basket. Basket that contains the organ tissue are placed in an automatic processor machine. Furthermore, the network experienced a gradual dehydration process with a lap time as follows: ethanol 70% (2 hours) ethanol 80% (2 hours), 90% ethanol (2 hours) 96% absolute ethanol (2 hours) xylol (2 hours) of liquid paraffin (2 hours). Furthermore, the basket which contain tissue cassette is issued to do the next process.

3. Vacuum

Dehydration process carried out, followed by removal of air from the tissue using a vacuum machine that includes the tube to store basket filled with liquid paraffin with temperature (59-60 ° C) in a vacuum for 30 minutes. Basket removed, the tissue cassette removed and stored at 60 ° C for a while before printing done with liquid paraffin.

4. Paraffin Block Production

Prepare some iron mold that has been smeared with glycerin in order to prevent the paraffin sticking inside the mold, then a tissue that has been cut included with tweezers and wait until the paraffin solidified. Paraffin blocks removed from the mold and stored in a freezer (-20 ° C) before cutting. Parafn block containing the tissue was cut using a microtome machine with thicknesses ranging from 3-4 µm.

5. Tissue Block Slicing

The tissue blocks pieces are carefully placed on the surface of water in a water bath at temperature 46 ° C. On this occasion the tissue sliced orderly then placed on a microscope slide that smeared with ewith, which serves as an adhesive. Slide with tissue on it are arranged in a special rack and put in an incubator at 60 ° C until the specimen is ready for staining process.

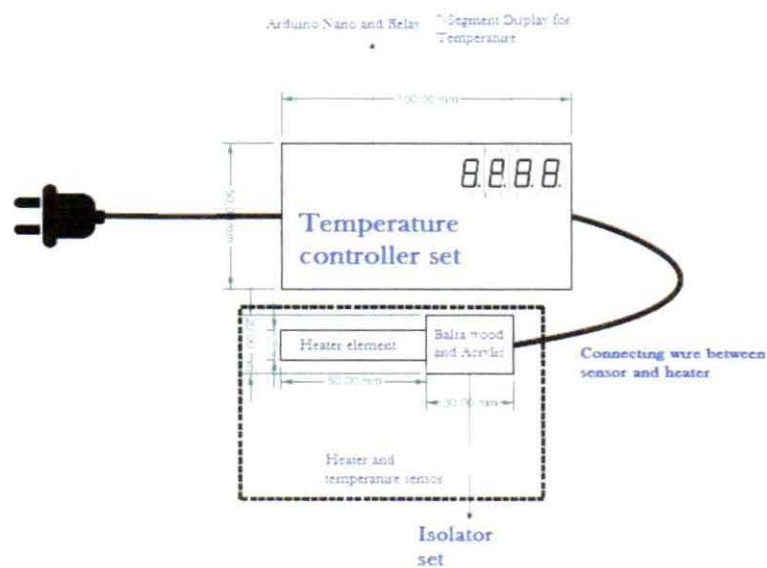
6. Hematoxilin – Eosin Staining

Specimen which was stained are placed in a special rack then dipped in a series into some solutions with time as follows :

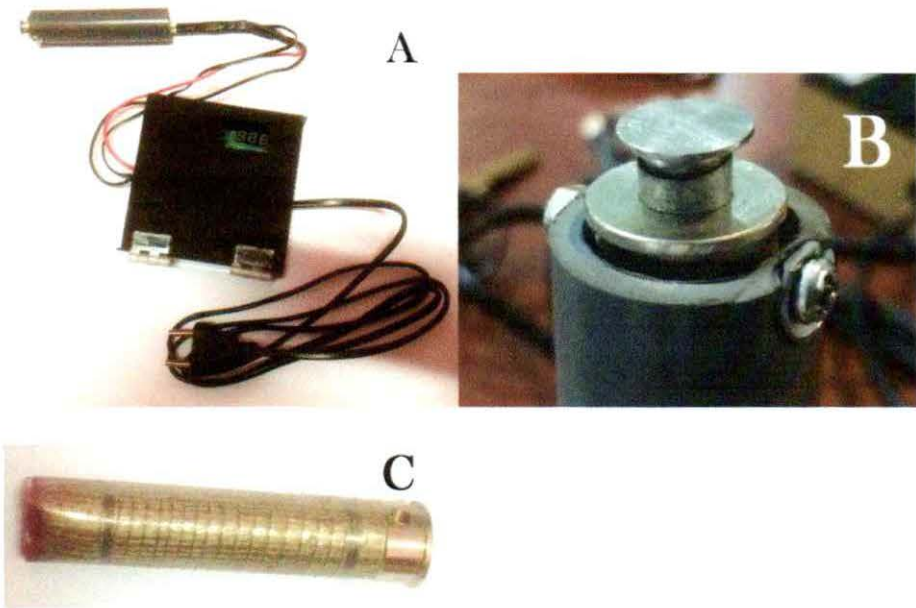
- | | |
|-----------------------------------|-----------------------------------|
| 1. Xylol 3 minutes | 12. Eosin solution 5 minutes |
| 2. Xylol 3 minutes | 13. Rinse with tap water 1 minute |
| 3. Absolute ethanol 3 minutes | 14. Ethanol 80% 10 times dip |
| 4. Absolute ethanol 3 minute | 15. Ethanol 90% 10 times dip |
| 5. Ethanol 90% 3 minutes | 16. Absolute ethanol 10 times dip |
| 6. Ethanol 80% 3 minutes | 17. Absolute ethanol 1 minute |
| 7. Rinse with tap water 1 minute | 18. Xylol 3 minute |
| 8. Hematoxilin solution 7 minutes | 19. Xylol 3 minute |
| 9. Rinse with tap water 1 minute | 20. Xylol 3 minute |
| 10. Blue solution 1 minute | |
| 11. Tap water 1 minute | |

Specimens removed one by one from xylol solution in wet condition, then give one drop of liquid adhesive and then covered it with cover glass. Staining results can be seen under a microscope using drops of oil emersion to clarify the examination (Muntiha, 2001).

Appendix 3 Modified Thermostat for Making Burn Wound



Gambar 1. Modified thermostat scheme



Gambar 2. Alat thermostat modifikasi, (a) Full set of modified thermostat, (b) Stainless plat, (c) Heater.

Appendix 4 Result of Histopathological Observation



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HASIL PEMERIKSAAN HISTOPATOLOGI

Nama Pemilik : Sis Wisnuarti Theresia
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Pemeriksa : Djoko Legowo, drh., M.Kes

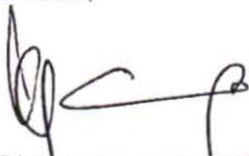
Metode Pemeriksaan

Metode perhitungan jumlah fibroblas pada pemeriksaan ini dilakukan menurut metode Fuadi *et al.*, (2015) dengan modifikasi. Penghitungan ini dilakukan dengan mikroskop cahaya merk Nikon ECLIPSE E100LED MV R dan OPTILAB pada lima lapangan pandang secara sistematis pada perbesaran 400x. Perhitungan dilakukan dengan metode blinding kemudian dari lima lapangan pandang fibroblast yang teridentifikasi dijumlah dan dicari rata-ratanya.

HASIL PERHITUNGAN FIBROBLAS PADA LUKA BAKAR DERAJAT II B

Kode Preparat	Jumlah Fibroblas per Lapangan Pandang					Rata-rata	Rata-rata per Perlakuan
	1	2	3	4	5		
C- 1	9	10	13	8	14	10.8	11.44
C- 2	10	12	15	15	9	12.2	
C- 3	17	9	7	9	14	11.2	
C- 4	14	14	15	13	14	14	
C- 5	11	11	7	4	12	9	
C+ 1	24	21	39	41	30	31	35.72
C+ 2	45	32	42	38	28	37	
C+ 3	36	43	54	21	23	35.4	
C+ 4	39	37	27	28	54	37	
C+ 5	33	26	38	53	41	38.2	
T1.1	35	34	49	31	17	33.2	27.56
T1.2	24	15	25	46	26	27.2	
T1.3	25	27	29	32	27	28	
T1.4	29	24	18	24	19	22.8	
T1.5	23	28	15	47	20	26.6	
T2.1	47	54	34	33	53	44.2	41.76
T2.2	44	59	44	51	41	47.8	
T2.3	39	34	33	31	45	36.4	
T2.4	26	53	32	74	46	46.4	
T2.5	19	35	45	41	30	34	
T3.1	47	51	25	33	51	41.4	47.4
T3.2	42	55	42	70	71	56	
T3.3	30	24	53	51	50	41.6	
T3.4	39	32	62	70	72	55	
T3.5	36	27	58	41	53	43	

Surabaya,
Pemeriksa,


(Djoko Legowo, drh., M.Kes)
NIP. 196712141996031004

Appendix 5 Result Analysis

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
C-	5	9.00	14.00	11.4400	1.84065
C+	5	31.00	38.20	35.7200	2.81993
T1	5	22.80	33.20	27.5600	3.73202
T2	5	34.00	47.80	41.7600	6.18288
T3	5	41.40	56.00	47.4000	7.42832
Valid N (listwise)	5				

Case Processing Summary						
	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
C-	5	100.0%	0	0.0%	5	100.0%
C+	5	100.0%	0	0.0%	5	100.0%
T1	5	100.0%	0	0.0%	5	100.0%
T2	5	100.0%	0	0.0%	5	100.0%
T3	5	100.0%	0	0.0%	5	100.0%

Tests of Normality						
	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
C-	.164	5	.200*	.989	5	.976
C+	.275	5	.200*	.841	5	.167
T1	.253	5	.200*	.944	5	.691
T2	.253	5	.200*	.878	5	.299
T3	.323	5	.096	.758	5	.036

*. This is a lower bound of the true significance.
a. Lilliefors Significance Correction

Oneway

Test of Homogeneity of Variances

Fibroblast			
Levene Statistic	df1	df2	Sig.
7.795	4	20	.001

ANOVA

Fibroblast

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3910.554	4	977.638	40.213	.000
Within Groups	486.224	20	24.311		
Total	4396.778	24			

Post Hoc Tests

Homogeneous Subsets

Fibroblast

Duncan

Ulangan	N	Subset for alpha = 0.05			
		1	2	3	4
Control Negative	5	11.4400			
Treatment 1	5		27.5600		
Control Positive	5			35.7200	
Treatment 2	5			41.5600	41.5600
Treatment 3	5				47.4000
Sig.		1.000	1.000	.076	.076

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Appendix 6 Certificate of Ethical Clearance


**UNIVERSITAS AIRLANGGA FACULTY OF DENTAL MEDICINE
HEALTH RESEARCH ETHICAL CLEARANCE COMMISSION**

ETHICAL CLEARANCE CERTIFICATE
Number : 307/HRECC.FODM/XI/2018

Universitas Airlangga Faculty Of Dental Medicine Health Research Ethical Clearance Commission has studied the proposed research design carefully, and therefore, shall herewith certify that the research entitled :

“THE EFFECT OF GIVING GEL COMBINATION OF BINAHONG LEAF EXTRACT AND TURMERIC RHIZOME EXTRACT ON THE COLLAGEN DENSITYVOF RATS WHICH HAVE II B DEGREE BURNS”

Principal Researcher : IGNASIA FRISKA AMELIA S

Unit/Institution/Place of Research : - Fakultas Kedokteran Hewan Universitas Airlangga Surabaya

CERTIFIED TO BE ETHICALLY CLEARED



Surabaya, November 22th, 2018
Chairman,



Prof. Dr. M. Rubianto, drg.,MS.,Sp.Perio(K)
Official No.195009081978021001

Appendix 7 Documentation

Burnazin (Silver Sulfadiazine)



Binahong Leaf Powder



Xylazine and Ketamine



Wistar strain white rat as
experiment animal



Combination Gel of
Binahong Leaf Extract and
Turmeric Rhizome



Sampling Process



Turmeric Rhizome Powder



Minor Surgery Tools