Utilization of Haramounting Nano- Herbal(Rhodomyrtustomentosa) In Burn Wound Healing II in Rats

Elidawaty Silaban¹, I NyomanErlich Lister¹*, Edy Fachrial¹
¹(Study Program of Biomedical Science Master, Medical School, Universitas Prima Indonesia

Abstract
Background: There are reports that haramounting has antioxidant and antibacterial properties. Therefore, it can be developed as an ingredient in accelerating wound healing. Because the wound will cause increased oxidants and invite the presence of bacteria or other microorganisms to grow and will certainly inhibit the wound healing process.

Materials and Methods: This study uses a true experiment method with a completely randomized design, four treatments (negative control, positive control, bioplasenton, haramountingnano-herbal), and observation time of burns healing (0 days, 4, 8, 12 and 16 days) with each field four replications.

Results: It was found that haramountingnano-herbal was very effective in healing second degree burns in rats for 16 days without leaving Gram (-) and Gram (+) (effective antibacterial) bacteria. Bioplasenton is effective in healing second-degree burns in rats for 16 days, but the antibacterial properties still leave Gram (+) bacteria.

Conclusion: The effectiveness of healing degree II burns in mice is better in haramountingnano-herbal than bioplasenton as well as its antibacterial properties.

Key Word: Nano-herbal, haramounting, burn wound healing.

I. Introduction

The degree of burn is divided into 4 namely burns degree I, IIa, IIb, III. Second-degree burns are frequent burns. Skin affected by burns will experience damage to the epidermis, dermis, and subcutaneous tissue. This depends on the factors that cause burns and the length of skin in contact with heat sources. The depth of the burn is influenced by the length of skin in contact with the heat source [1].

Efforts to heal burns can be done using chemical or herbal medicines. Examples of chemical drugs are for example haramountingnano-herbal. The advantage of bioplasenton in healing burns faster. Bioplasenton can also be used in wounds infected by bacteria, because the bioplasenton drug is in the class of antibacterial therapy. People with eczema wet or can also be said that long-lasting skin ulcers can use bioplasenton drugs. Skin disease in the form of impetigo that quickly spreads to the skin area can also use bioplasenton drugs. Bioplasenton deficiency is contraindicated in people who are allergic or hypersensitivity to the bioplasenton content. Patients or consumers who are found to have allergies or hypersensitivity to placental extracts and neomycin sulfate should not use bioplasenton drugs without doctor's approval. Unlike chemical drugs, herbal medicines in addition to accelerating the healing of burns, also do not cause allergic effects. But for standardization, it requires in-depth and continuous research until it can be used by humans [2]

Burns as an external factor include mechanical trauma (physical damage to the body that causes cellular damage), damage to blood vessels (which can interfere with blood supply to related tissues), and ischemia. Thermal effects (very high or low temperatures) can cause necrosis due to cell disruption. Under extreme conditions tissue and cells die through the process of destroying the unregulated membrane and cytosol. Necrosis can be activated by immune system components, such as the complement system; bacterial toxins (infections), activated natural killer cells, and peritoneal macrophages. Toxins and pathogens can cause necrosis and can inhibit enzymes and cause cell death. In addition bacterial cells and secretions from a subset of bacterial species inhibit migration of human and pig epithelial cells in vitro and ex vivo. Some bacteria that play a role in burn infections include Serratiamarcescens, Pseudomonas aeruginosa, and strains of Staphylococcus aureus, Klebsiella pneumonia, Candida albicans, and Pseudomonas aeruginosa. Some bacteria produce lipopoliskarida (LPS) which inhibits the migration of fibroblast cells thereby slowing the healing of burns [3].

Researchers have concluded that caramunting leaf extract has the potential to be used as an antibacterial. At the optimum concentration of ethanol extract of caramunting leaves in inhibiting the bacteria
Utilization of Haramounting Nano-Herbal (Rhodomyrtustomentosa) In Burn Wound ...

Shigellasp and Staphylococcus aureus was 80%. The results of other studies showed that the antibacterial activity of methanol extract, ethyl acetate fraction, and n-butanol fraction of karamunting roots against Escherichia coli and Staphylococcus aureus bacteria had a growth inhibition response included in the medium category with inhibition zones and killing zones between 5-10 mm. The data obtained were processed using one-way analysis of variance and continued with the HSD test (honestly significant difference) on the extract and fraction of karamunting root. The results of the analysis showed that the best concentration of extracts and fractions of karamunting roots against both test bacteria was 50% [4], [5].

There are research reports on the antioxidant activity of haramounting to prevent ROS from causing deathl [6]. But there is still very little information related to the biological activity of haramountingnano-herbal, especially in increasing antioxidant activity related to prevention or accelerating the healing of burns on the skin. It is hoped that these data can support the development of haramountingnano-herbal as an active ingredient in the prevention of burns to the skin.

II. Material And Methods

Research Samples: The research sample of haramounting plants (Rhodomyrtustomentosa) was obtained at Padang Sidempuan, North Sumatra and identified at Medanese Herbarium (MEDA), Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Sumatera Utara (USU), Medan. Rat samples were from the Animal Cage Department of Biology, FMIPA, USU, Medan. Male rat (Rattusnorvegicus), 8-11 weeks old, weighs 150-200 g, healthy and fed CP551 pellet and drank adlibitum. Before the study was obtained an Ethical Clearance Letter from the Animal Research Ethics Committee, FMIPA - USU.

Research design: This pure experimental research was carried out with a completely randomized design (CRD) with haramounting leaf haramountingnano-herbal test used 100 mg dissolved in 2 mL NaCl 0.9%. There are 4 treatment groups namely:

Table no 1 :Groups of treatment

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment group</th>
<th>Observation parameters (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>C(-)</td>
<td>X</td>
</tr>
<tr>
<td>2.</td>
<td>C(+)</td>
<td>X</td>
</tr>
<tr>
<td>3.</td>
<td>Bioplacenton</td>
<td>X</td>
</tr>
<tr>
<td>4.</td>
<td>Haramountingnano-herbal</td>
<td>X</td>
</tr>
</tbody>
</table>

Haramountingnano-herbal (Rhodomyrtustomentosa): Haramountingnano-herbal are made from dried simplicia of haramounting leaves using a high energy milling (HEM) tool. All work is done at LIPI Serpong Jakarta. The procedure for making haramountingnano-herbal is as follows: Insert the balls in the form of a device from the HEM tool as a crushing media into the jar container, by means of balls with a larger diameter inserted first and then proceed by inserting smaller balls and last sample. The total volume of the balls and the sample put in the jar does not exceed 2/3 of the jar volume. BPR (Ball to Powder Ratio) commonly used is 20:1; 10: 1; 8: 1. For example BPR 20:1 means that 1 gram of sample is milled with a ball weight of 20 grams. Furthermore, the jar which contains the ball and the sample is closed tightly, and installed on the jar holder contained in the HEM tool, then the HEM is ignited so that it takes a lap for 2 hours [7].

Testing the Burn Healing Effects: Testing the healing effect of burns is done by using male mice (Rattusnorvegicus). Mice shaved on the back. The skin is induced by a heat induction tool which has a temperature of 80 °C for 5 seconds. A heat induction tool in the form of a soldering iron at the end is affixed to a metal plate measuring 10x10 mm2. The wound is measured, then smeared with drugs according to each treatment group (according to the research plan), then the extent of the wound is observed until the wound heals (the wound shrinks and finally closes). The percentage of burn wound healing is calculated from the area of the wound that formed on each observation day (days 4, 8, 12 and 16).

Microbial content on the wound surface: Cotton is taken from the burn site on days 0, 4, 8, 12, and 16. (1) Sample Collection Stage: At the observations of days 0, 4, 8, 12, and 16th, microbial samples of rat burns were taken by means of rub cotton bud on the surface of the burn. Then put into white plastic and put a mark. (2) Media Making Stage: Making nutrient media to make 39 g in 1000 mL of distilled water and then sterilized using an autoclave at 1210°C for 15 minutes. (3) Dilution Stage: Samples in the cotton bud are put into 10 mL of physiological NaCl solution and then homogenised using vortex. Then take 1 mL of sample into a 10-1 dilution factor and homogenize it. Next insert a 1 mL sample from the 10-1 dilution factor to the 10-2 dilution factor, and do the same with the 10-3 dilution factor [8]. Dilution factors of 10-1, 10-2, and 10-3 each contain a physiological NaCl solution of 9 mL. (4) Isolation Phase: The isolation stage is carried out using the pour method, which is 0.1 mL for each dilution factor that is poured into the cup before being given agar nutrient.
media. Microbial isolation from rat burn samples was carried out in duplicate with dilution factors of 10-1, 10-2, 10-3, 10-4 and 10-5. After that the sample was isolated and incubated at room temperature 25-27°C for 24 hours. (5) Observation Stage. Microbial colonies growing in each sample cup were counted using a colony counter, the number of microcolonies analyzed was a range of 30-300 cfu/mL colonies (Sukmawati, 2018). The microbial calculation of the planted dilution is 10-4 cfu/mL and 10-5 cfu/mL. Bacteria per ml = Number of CFU / Dilution volume (ml) x total dilution used [8]

**Determination of the types of microbes on the wound surface:** To determine the type of bacteria carried out by bacterial culture, bacterial morphology, Gram staining and planting on identification media. Bacterial culture is the growth of bacteria from microorganisms. Microorganisms grow in a medium that consists of substances that stimulate the growth of microorganisms that are suspected as causes and inhibit unwanted microorganisms. Material suspected to contain microorganisms is etched on the surface of the media then the plates are incubated at the appropriate temperature. After that, bacterial growth and colony morphology were observed [9]

**Isolation and identification of bacteria:** Isolation and identification of *Staphylococcus aureus* and *Streptococcus* sp. Samples were taken with a sterile cotton bud and were grown on a T-method sheep blood agar plate (PAD) and incubated at 37°C for 24 hours. Gram staining, Mannitol salt agar Mannitol salt agar (MSA). Coagulase test, Test Voges Proskauer. Confectionery. Isolation and identification of *Pseudomonas bacteria*. Gram staining is done. Pseudomonadaceae genus *Pseudomonas*. Characteristics selected by morphology, stem shape, motile due to flagella, and gram negative. Growth is aerobic, and growth temperature is 4-43°C. A total of 1 ml of each dilution was mixed with Pseudomonas Isolation Agar (PIA) selective media on a petri dish. Then incubated at 37°C for 48 hours in an upside down petri dish position. After incubating the bacterial growth was observed. Separate colonies were transferred using an ose needle into the media to tilt Casamino Acid Media. Then incubated for 48 hours to obtain a single bacterium (pure isolate). Pure isolates produced from bacterial culture, bacterial morphology, Gram staining and planting on identification media. Bacterial culture is the growth of bacteria from microorganisms. Microorganisms grow in a medium that consists of substances that stimulate the growth of microorganisms that are suspected as causes and inhibit unwanted microorganisms. Material suspected to contain microorganisms is etched on the surface of the media then the plates are incubated at the appropriate temperature. After that, bacterial growth and colony morphology were observed [9]

**Data analysis:** Data from the identification of the phytochemical content of harount is arranged in the Table. For numerical data on the test parameters will be analyzed with one way ANOVA bootstrapping, if p<0.05, then proceed with Mean Comparison of Group - Post Hoc Duncan analysis at 5% level.

### III. Result

Based on the results of research conducted at the Laboratory of the Faculty of Medicine, Hospital of the University of North Sumatra and the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, it can be explained as stated below.

**Second degree burns area:** To evaluate the healing effects of second-degree burns in mice an observation of the percentage of burn wound was performed. Observation data obtained by the data as shown in Table 2 below.

### Table no 2: Percentage of grade II burn closure in rats during healing (%).

<table>
<thead>
<tr>
<th>Group</th>
<th>Healing Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>C(-)</td>
<td>0.00</td>
</tr>
<tr>
<td>C(+)</td>
<td>0.00</td>
</tr>
<tr>
<td>Bioplacenton</td>
<td>0.00</td>
</tr>
<tr>
<td>Haramountingnano-herbal</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Type of bacteria:** Observation of the types of bacteria that are well identified can be noted in Table 3 below.

### Table no 3: Types of Gram negative and Gram positive bacteria in healing wistar degree II burn wounds.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Duration of wound healing (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>C(-)</td>
<td>Pseudomonas</td>
</tr>
</tbody>
</table>

DOI: 10.9790/3008-1504034651  www.iosrjournals.org 48 | Page
Utilization of Haramounting Nano-Herbal (Rhodomyrtus tomentosa) In Burn Wound

Bacterial count: The results of the study have obtained the number of bacteria in each observation of wound healing time as shown in Figure 1 below.

**Figure 1.** Average number of colonies (CFU / mL) of bacteria during degree II burn healing.

**Table no 4:** The number of bacteria (-) and Garm (+) at some time healing rat burns.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Second degree burn healing time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>C(-)</td>
<td>23.00±4.24</td>
</tr>
<tr>
<td>C(+)</td>
<td>16.00±1.41</td>
</tr>
<tr>
<td>Bioplacemonton</td>
<td>11.00±1.41</td>
</tr>
<tr>
<td>Haramountingn ano-herbal</td>
<td>8.00±1.41</td>
</tr>
</tbody>
</table>


IV. Discussion

The percentage of closure burn wound II in rats on the first day of burns (day 0) is 0.00 because new wounds are formed and have not been seen and when measured there has been no change in the closure of burns. Physiologically the inflammatory process has occurred as an initial stimulus from cells to the incidence of second-degree burns. The inflammatory process can be stimulated by chemical factors (histamine, bradykinin, serotonin, leukotrienes, and prostaglandins) released by cells that act as inflammatory mediators in the immune system to protect surrounding tissues from spreading pathogenic microbial infections.
The percentage of wound healing occurred on day 16 is 99.81%. This figure shows that the percentage of healing in haramountingnano-herbal treatment is better than the use of bioplasenton which is only 32.14%. There are reports stating that Rhodomyrtusustomentosa methanol extract can have anti-inflammatory properties by suppressing NF-kB and will be further developed as an herbal medicine for the prevention and / or cure of various inflammatory diseases [10], [11].

The types of bacteria found during the observation of second-degree burns during the burn healing period consisted of 7 types of bacteria that were recorded starting from 0, 4, 8, 12, and 16 days namely from Pseudomonas sp., Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Streptococcus sp., Klebsiella sp., Klebsiellapneumoniae. The type of bacteria increased from the beginning of observation until day 4 and then decreased at the end of the observation (day 16). But specifically for treatment with haramountingnano-herbal that the type of bacteria at the end of observation (day 16) no longer found bacteria or free from bacterial life. In contrast to bioplasenton which is still found by Streptococcus sp. at the end of the observation (day 16).

Haramounting nano-herbal contains flavonoids which are reported to have anti-microbial action. It has been reported that Rhodomyrtustomentose and rhodomyrton leaf extracts show promising antibacterial activity against S. pyogenes clinical isolates. [5]. Rhodomyrtustomentose leaf can be used to treat bacterial (antibacterial) infectious diseases especially infections of S. aureus and P. aeruginosa. Rhodomyrtustomentose leaves are analyzed for phytochemical compositions such as alkaloids, flavonoids, and tannins. Phytochemical screening of crude ethanol extract, hexane fraction, and ethyl acetate fraction of Rhodomyrtustomentose leaf powder recorded a very high content of flavonoids and tannins in the ethanolic and ethyl acetate fractions. The high values noted for plant extracts show that Rhodomyrtustomentose powder from leaves can be a good antimicrobial source, especially as an antibacterial agent [4].

In Figure 1 it can be seen that the number of bacterial colonies found in second-degree burns of rats initially increased until day 8 and began to fall on day 16. The number of bacterial colonies in the P2 group (administration of haramountingnano-herbal) was not as much as other treatments, and at day 16 no more bacteria were found. This indicates the chemical content of nanoharamounting herbs such as flavonoids can act as antibacterial in rat second degree burns. As a research report which states that phenolic compounds and flavonoids can be used as an antibacterial agent [6], [12]–[14].

The results of observations of the number of Gram (-) and Gram (+) bacteria initially (day 0) are fewer and increase on observations of days 4 and 8, on days 12 and 16 begin to decrease in number. There is a large decrease in the administration of bioplasentons, including in the administration of haramountingnano-herbal which is no longer found Gram (-) and Gram (+) bacteria. This proves that haramountingnano-herbal nano-herbal is better in suppressing the presence of Gram (-) and Gram (+) bacteria in second-degree burns. The presence of antibacterial chemical compounds in haramountingnano-herbal causes inhibition and death of Gram (-) and Gram (+) bacteria in burns. According to studies that have been reported that haramountingnano-herbal contains flavonoids and polyphenols which can act as antibacterial [6], [14]–[17].

V. Conclusion

Based on the results and discussions that have been carried out in the study of the effects of haramountingnano-herbal on second-degree burns, the following conclusions and suggestions can be drawn;
- Haramountingnano-herbal is very effective in healing second degree burns in rats for 16 days without leaving Gram (-) and Gram (+) bacteria (effective antibacterial).
- Bioplasenton is effective in healing second-degree burns in rats for 16 days, but the antibacterial properties still leave Gram (+) bacteria.
- Effectiveness of healing degree II burns in mice is better on haramountingnano-herbal than bioplasenton as well as its antibacterial properties.

References

[7]. C. Fatimah, Universitasumaterautara, I. Medan: Disertasi S3 FarmasiFakultasFarmasiUniversitas Sumatera Utara, 2018.

DOI: 10.9790/3008-1504034651 www.iosrjournals.org 50 | Page
Utilization of Haramounting Nano-Herbal (Rhodomyrtustomentosa) In Burn Wound Healing In Rats.


DOI: 10.9790/3008-1504034651 www.iosrjournals.org 51 | Page