

Effects of Gambir (*Uncaria gambir* Roxb) Catechins on Burn Wound Healing in Male Rats

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Abstract: Background: Indonesia is the largest *Uncaria gambir*, Roxb (*gambir*) producing country in the world, but its benefits as a medicinal plant has not been optimally developed. Traditionally, *gambir* plants have been widely used by a lot of people as burn wound healing. Objective: This study aims to determine the effect of *gambir* catechins crude for burn wound healing through histopathological observations on white rats male. Methods: *Gambir* Catechins crude was isolated by using partition method with ethyl acetate solvent. The rats were made to suffer burn wound based on Akhoondinasab methods et al. A total of 30 male white rats were divided into 5 groups and given daily test preparations. A total of 3 groups were given *gambir* catechins crude gel with variations of concentrations of 1%, 2% and 4%), for positive control group was used a commercial drug that contains 10% placenta extract and 0.5% Neomycin sulfate and one other group was used as a negative control. After administration of test preparation for 21 days, histopathology changes was observed in the burn wound that occurred. Results: Statistical analysis showed that gel *gambir* catechins crude with concentrations 2% had effect of decreasing the burn wound area and an increase in the percentage of healing of burn wound that did not differ significantly from positive controls ($P < 0.05$). Histopathologic observations showed that catechins crude of *gambir* were able to reduce the number of inflammatory cells and increase neocapillary formation compared with the negative control group. Conclusion: *Gambir* catechins crude with concentration 2 % could help the healing process of second degree of burn wound on the inflammatory phase and proliferation.

1 INTRODUCTION

Burn wound is one of the most common and devastating forms of trauma and a major health problem of morbidity and mortality worldwide, because burn wound is very frequent cause of infection and difficult to cure and often cause scarring on the burn wound tissue (Kaddoura et al 2017, Church et al 2006). This greatly reduces the value of skin beauty and the price of these drugs is very expensive (Rowan et al, 2015). Current methods to treat burn wound include antibiotics, antiphlogistics, and silver salt, which possesses major drawbacks and unwanted side effects, (Waserman, 2002, Atiyeh et al, 2007) Therefore it is necessary to find a healing drugs burn wound that does not cause scarring on the burn wound tissue and the price is cheaper. Generally drugs that come from natural ingredients have smaller side effects. One of the natural ingredients that have been proven to be better and safer for burn wound healing than

synthetic drugs that are widely used today (1% silver sulphadiazine cream) is Aloe Vera Gel (Shahzad and Ahmed, 2013)

According to the research result of Hananeh et al (2015) the use of propolis cream was the best overall wound healing effects in full thickness skin wounds in rats, where in this study, the inflammatory reaction was weaker in the propolis treated group than in the other groups treated with silver sulphurdiazine (SSD) and bepanthane (Hananeh et al 2015). Besides that *Centella asiatica* also has a very good effect on burn wound healing (Somboonwong et al, 2012)

On the other hand, the use of honey as a topical treatment of wounds in diabetic patients in both human and veterinary medicine, revealed that both natural honey and amikacin enhanced wound healing in non -diabetic rat patients. Honey showed more promising results compared to amikacin in enhancing the healing of full-

thickness punch biopsy wounds in diabetic rats (Hananeh et al 2015)

Gambir (*Uncaria gambir*, Roxb) is a kind of herbal that has been known in Indonesia and other countries for a long time. Gambir is added on betel nut chewing as well as additional substance in traditional herbal medicine. Traditionally gambir has been used for people with diarrhea, sore throat, burn wound healing, anti-cancer, immunomodulator, etc (Murdja et al 2017, Li et al, 2013)

The main content of the gambir is catechins (up to 51%), tannin (22-50%), and a number of alkaloids such as gambirtannin, derivatives dihydro and oxo of gambirtannin. There are 9 types of catechins that were found of gambir, namely; (+)-Catechin, (+)-epicatechin, Gambiriin A1, Gambiriin A2, Gambiriin B2, Epigallocatechin, Catechin-(4 α -8)-ent-epicatechin, Gambirflavan D1 and Gambirflavan D2 (Taniguchi et al, 2008).

According to research of Murdja et al (2017), "The (+)-Catechin was the highest level among 9 types of catechins with concentration about 45.66% of the total catechins in gambir. Therefore (+)-catechin can be used as a biomarker to determine the quality of gambir. Therefore in this study only (+)-catechin (biomarkers) was measured by a spectrophotometer UV-VIS at wavelength 279 nm" (Murdja et al, 2017, Isnawati, 2010)

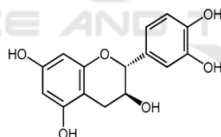


Figure 1. (+)-Catechin, main content of gambir (Murdja et al, 2017)

Extract of gambir could inhibit the growth of *Streptococcus mutans*, *Staphylococcus aureus* and *Bacillus subtilis* extract of gambir also could inhibit the growth of *Helicobacter pylori* that resistant to antibiotic (Heitzman et al, 2005, Voravuthikunchai, and Mitchell 2008). The effect of gambir as an antimicrobial is thought to prevent infection in burns wound, so it will accelerate on burn wound healing (Mohamed et al, 2008)

In addition, traditionally, a lot of people in Indonesia have long used gambir as a drug for burn wound healing. Based on this reason, we conducted a study on the effect of gambir catechins on burn wound healing on male rats.

2 METHODS

Gambir was obtained from Payakumbuh region, West Sumatra, the largest producer of gambir in Indonesia, to ensure that the sample used was *Uncaria gambir Roxb*, in this research was done determination of plant taxonomy in Biological Research Center of Indonesian Institute of Sciences, Bogor, Indonesia.

Phytochemical screening of gambir extract was done based on Harbone methods. (Harborne, 1998, Farsworth, 1969).

Catechins isolation from gambir extract was done based on National Standard Indonesia: SNI 01-3391-2000, "A total of 500 g of gambir powder was extracted with a water solvent at temperature of 90 - 96 0C for 15 minutes while stirring. Then the infusion was filtered in hot conditions using a funnel coated with filter paper. The extract obtained was partitioned with ethyl acetate, the ratio of extract with ethyl acetate (1 : ½). Ethyl acetate phase was taken and the water phase was partitioned repeatedly with ethyl acetate until a clear solution was obtained. The ethyl acetate phase was condensed with an evaporator, then washed with cold water and filtered. Catechins that obtained was dried in an oven at temperature 70 °C". Then, The quality of gambir extract was determined based on National Standard Indonesia: SNI 01-3391-2000. In this research, the yield of catechins obtained from the gambir extract was determined as (+)-catechins by comparing with standard (+)-catechins and measured by a spectrophotometer UV-VIS at wavelength 279 nm. (Ferdinal, 2014, Hargono, 1986).

The yield catechins from gambir calculated by the formula:

$$\% \text{ Yield} = \frac{\text{weight of acquired isolate}}{\text{weight of extracted powder}} \times \% \text{ purity}$$

Preparation of catechins gel was made base on modification of (Eva et al, 2014, Rini et al 2013) methods. In this experiment, crude catechins of gambir was mixed into gel formulations as below.

Na CMC	3,75%
Propilen glikol	3,75%
Gliserin	7,5%
Nipagin	0,05%
Aquadest	ad 60

The crude catechins of gambir in the gel formula was prepared with concentrations : 1%, 2% and 4% b/w . Evaluation of this formulation was done by determination: organoleptic, homogeneity, viscosity and pH of the gel formula based on the provisions of the Drug and Food Control Agency, RI (Ditjen POM RI, 2000).

Experimental Animal was used male white rats (*Rattus novergicus*) Sprague Dawley strain, aged 2-3 months with a weight of 150-190 gram. Animals were acclimatized for 1 week in order to adaptation to the environment and during the adaptation process was carried out observations of general conditions and animals weight were weighed every day. Animals that qualify for the experiment were selected (Hoekstra et al, 1993, Amini et al, 2010).

Burn wound was made based on a method undertaken by Akhoondinasab et al, 2014. Testing the effects of burn wound healing was done on 30 rats. The feather on the dorsal area about 3 cm from the ear of the rats was shaved and given local anaesthesia with ketamine. Burn wound induction process was performed by using a 4x2 cm iron plate heated for 5 minutes in boiling water and then attached to the skin of the back for 10 seconds at the same pressure (Akhoondinasab et al, 2014).

Animals were divided into 5 groups and each group consisted of 6 rats, each group was used for testing, positive control, negative control, gambir catechins crude with doses : 1%, 2% and 4% b/w.

The burns wound were observed and measured, and then smeared the drug according to each group, namely, negative control with gel base, positive control with commercial drug that contains 10% placenta extract and 0.5% Neomycin sulfate and three groups with crude catechins of gambir with concentration of 1%, 2% and 4%.

Giving test gels were done topically as much as 0.2 g for 1x polishing by applying it on the burn wound in each group of treatment rats. Giving test gels were done every day, from day 1 to day 21, 2 times a day, in the morning and evening.

Histopathological observations were done in daily from day 1 to day 21 after burns wound to all treatment rats. Observation was done by looking directly at the burn wound healing. To assess burn wound healing, photographs obtained were processed with Image software and calculated the percentage of healing (Akhoondinasab et al, 2014).

Skin tissue samples were taken on the 7th day, from each of the five groups were taken 1 rat. Skins retrieval were carried out after rats were euthanized by using ether solution per inhalation. The area skins of the back would be taken were cleansed from the hair that starts to grow, the skins were cut with a thickness of ± 3 mm to the sub-cutaneous with area of 1-1.5 cm². The skins obtained were fixed with a 10% Formalin Neutral Buffer solution and left at room temperature for ± 48 hours (Kulac et al 2013, Prasetyo et al 2010).

The skin tissue obtained was made histopathological preparations with Hematoxylin-Eosin dye by the Histopathology team at the Pathology Laboratory, Faculty of Medicine, University of Indonesia.

Histopathologic observations were done on skin tissue preparations taken on day 7. The observations were done by descriptive light microscopy. These observations include the parameters that play a role in burn wound healing namely the presence of inflammatory cells and neocapillarization. (Jose et al 2010).

Histopathology Observation Scoring Reading was done by using Hosseini methods, (S.V. Hosseini et al 2011).

For measurement of percentage of wound healing, done by looking directly at the wound then measured the area of burn with image application and calculated the percentage of healing burns and calculated with formula in below :

$$\% \text{ Burn wound healing} = \frac{(\text{extent of initial wound-wound end})}{(\text{initial wound area})} \times 100\%$$

The test results data were analyzed using data processing software and presented in the mean and standard deviation of each group. The data were processed using statistical analysis with normality test, homogeneity test, One Way ANOVA and Kruskal-Wallis Test.

3 RESULT AND DISCUSSION

The result of determination of plant taxonomy in Biological Research Center of Indonesian Institute of Sciences, Bogor, Indonesia stated that the plant extract used in this research was an extract from *Uncaria gambir* Roxb.

The result of screening of chemical group content of gambir extract based on Harbone methods was shown in table 1. This was the same with the research results of Taniguchi et al (2008), that gambir extract contains groups of chemical compounds alkaloids, flavonoids, saponins, tannin and Quinone (Taniguchi et al, 2008).

Gambir quality that was used in this study, when compared with the provisions of the Indonesian National Standard: (Indonesian National Standard: SNI 01-3391-2000) as shown in table 2.

Gambir used in this research was number one quality, or fulfills the requirements for research.

Table 1. The content of groups of chemical compounds in the gambir extract that was studied

Chemical group	Results
Alkaloids	+
Flavonoids	+
Saponin	+
Tannin	+
Quinone	+
Steroids & Triterpenoids	-
Essential oil	-
Qoumarine	-

Catechins are flavonoid compounds that include natural phenolic compounds that are potential as antioxidants and have bioactivity as a drug (Lucida, 2007). The working mechanism of the flavonoid is to circulate blood throughout the body and prevent the occurrence of blockage in the blood vessels (Handayani, 2015). In addition, catechins are potentially antibacterial (Arakawa et al 2004) According to Anggraini et al (2011) flavonoid compounds have anti-inflammatory effects that serve as anti-inflammatories and are able to prevent stiffness and pain (Anggraini et al 2011).

Catechins have activity as antibacterial, especially gram-positive bacteria tested on Staphylococcus aureus bacteria where this bacterium is one of the gram-positive bacteria that often cause wound infection (Murdja et al 2017).

Table 2. Comparison of gambir quality that was used in this research with gambir quality requirements of National Standards of Indonesia (Standar Nasional Indonesia : SNI 01-3391-2000)

Testing of gambir extract	Quality number 1	Quality number 2	Gambir extract for experiment
a. Physical condition :			
shape	Intact	Intact	Intact
color	Yellow to brownish yellow	Yellow to brownish yellow-black	Yellow to brownish yellow
smell	Specific	Specific	Specific
b. Water content w/w			
	≤ 14%	≤ 16%	4%
	≤ 5%	≤ 5%	1.03 %
c. Ash content w/w			
	≥60%	≥50%	88.65%
d. Catechins concentration w/w of dry weight			
	≤ 7%	≤ 10%	3.9%
e. Insoluble material content of :			
water w/w of dry weight	≤ 12%	≤ 15%	8.6%
alcohol w/w of dry weight			

In addition, the presence of antibacterial activity can suppress pathogenic bacteria and prevent the growth of pathogenic bacteria in wounds so that wound healing can be accelerated (Kaddoura et al 2017).

Catechins act as antibacterials by the mechanism of binding to the peptide unit on the peptidoglycan component of the cell wall. The occurrence of such binding can disrupt the integrity of bacterial cell walls and cause leaks in Gram-positive bacterial cells (Pambayun et al, 2007).

Therefore, the presence of antibacterial activity in catechins prevents the possibility of infection in the event of injury. Moreover, catechins have activity as an antioxidant that works by breaking the lipid peroxidase chain that plays a role in free radicals that can bind certain substances and harmful to the body (Musdja et al, 2018) and can damage three types of compounds that are important to maintain cell integrity, namely: fat, especially unsaturated fatty acids, which are important components of cell membrane phospholipids; DNA, is a genetic device of cells; protein, plays many important roles such as enzymes, receptors, antibodies, and cytoskeleton matrix formers (Ambiyani and Winny, 2013) So the existence of antioxidant activity in catechins can avoid more severe cell damage.

After examination of gambir quality, catechin isolates were formulated in gel preparation form. The gel preparation was chosen because it has a cooling, cooling, moisturizing, easy-to-use water content, easy penetration to the skin, thus providing a faster healing effect according to the base used. So it is in accordance with the principle of the main handling of minor burns that is cooling the burning wound with water, where the gel content consists mostly of water (Ansel, 2005). Observation of gel preparation shown in Table

Tabel 3. Observation of Gel Preparations

Observation	Dose 1%	Dose 2%	Dose 4%
Color	Light brown	Brown	Brown
Form	Semisolid transparent	Semisolid transparent	Semisolid, Not transparent
Smell	Specific	Specific	Specific
Homogeneity	Homogen	Specific	Specific
pH	6,51	6,12	5,76
Viscosity	43600	56400	73000

As the mechanism of action of positive control containing 10% placenta extract is growing new tissue and for healing wounds, while neomycin sulfate 0.5% to prevent gram negative bacterial infection in the wound area (Niknejad, 2013)

The formation of a scab indicates the process of wound healing entering the early stage of proliferation. To observe the formation of scab, in the wound was seen a network of granulation characterized by the appearance of a scab. This scab serves to close the wound and prevent injury from further contamination by microbes. The release of

scabs signifies the growth of new cells on the skin, thus helping to accelerate the release of scabs and to close the wound edges (Niknejad, 2013)

In the positive control group, the average scab was formed on day 4 and off on day 11. In the negative control group, the average scab was formed on day 3 and off on day 14. In the low concentration test group (1%), the average scab formed on the 2nd day and off on the 11th day. In the medium concentration test group (2%), the average scab was formed on day 2 and off on day 12. In the group high concentration test (4%), the average scab formed on day 1 and off on day 14.

Table 4. Observation of Scab

	Average on the day to				
	PC	NC	C1%	C2%	C4%
Scab formation, on days to	4	3	2	2	1
Remove the scab, on days to	11	14	11	12	14

Note:

PC = Positive Control, NC = Negative Control,

C1% = Concentration Test 1%

C2% = Concentration Test 2%, C4% = Concentration Test 4%.

Based on the observation of the formation of the scab, it was obtained that the low concentration test group (1%) had the potential to accelerate wound healing time as the formation of the most rapidly formed scab, ie on the 2nd day and released on the 11th day was almost close to the positive control group compared to the concentration test group medium (2%) and high concentration (4%) and negative control. In high concentration tests (4%) initially could accelerate drying in the wound area but this drying triggers the formation of scab or dead tissue that was very hard and thick and attached tightly to the surface of the wound. This dead tissue could inhibit the distribution of the active substance and the absorption of the drug so that the wound lasts longer. The duration of the process of formation of new tissue resulted in the length of the healing period. Therefore the high concentration test group (4%) had the longest peeling time. In addition, it could be observed that the negative control group undergoes a long wound healing process seen from the time of formation of the scab and the time of loss

of the scab. This matter showed that gel base alone did not affect the acceleration of wound healing.

The area of the initial injury to the width of the wound the day after the wound was made and the extent of the final wound was the area of the wound on the day of observation.

The area of the initial wound to the calculation of the percentage of wound healing was the area of the wound the day after the rat was injured, after 24 hours wound stability occurs. A wound can be said to be healed if the wound area had undergone epithelialization thoroughly and no longer requires treatment. The result of measurement of the reduction of burn area on all treatment groups on the 1st day until the 21st day using Akhoondinasab injection method (Akhoondinasab, 2014) as shown in Table 5 and Figure 1.

Giving gambir catechins gel with various concentration levels (1%, 2% and 4%) had an effect on time and percentage of burn wound healing. The highest percentage of reduction of burn wound area (in Cm^2) on 8th day was in positive control (59.60%) followed by medium concentration (2%) was 44.02%. Based on statistical test of medium concentration test (2%) was significantly different with high concentration test group (4%) and did not significantly different with negative control, low concentration test (1%) and positive control.

The highest percentage of reduction of burn wound area on 15th day was obtained on positive control (95,76%) followed by medium concentration test group (2%) that was 88,32%. Statistical results

on the 15th day showed that the group of medium concentration test (2%) was significantly different with the high concentration test group (4%) and did not significantly different with the low concentration test group (1%), positive control and negative control.

Based on the percentage of reduction of burn wound area on the 21st day, the positive control group showed the highest result (100%) followed by medium-concentration (98.02%) and low concentration test (97.87 %), negative control (95,98%) and high concentration test (93,99%). This suggests that the gambir catechins gel on the low concentration test (1%) and the middle concentration test (2%) had high activity and were almost equal to the positive controls in the percentage of second degree burn wound healing. However, statistically the percentage reducing of burn wound area indicates that the data were normal, but not homogeneously distributed so that the data processing was followed by a non-parametric Kruskal-Wallis test. The statistical data of percentage reducing of burn wound area showed not significantly different ($p > 0.05$) in all groups, as shown in Table 5 and Figure 1.

Histopathological Observation was done by light microscope descriptively, based on Figure 2, at 20 field of view with 400 x magnification. Histopathologic observations preparations were performed by scoring system. The scoring method was done by using the Hosseini methods (Hosseini, et al, 2011) as shown in Table 6 and Figure 2.

Table 5. Results of Measurement Reduction of Burn Wound Area (in Cm^2)

	Days to :						
	1 day	8 days		15 days		21 days	
	Average wound area	Average decreased wound area	Average percentage (%) wound	Average decreased wound area	Average percentage (%) wound	Average decreased wound area	Average percentage (%) wound
Concentration 1%	7,34	3,02 ± 0,86	41,14	5,90 ± 1,01	85,83	7,18 ± 0,77	97,87
Concentration 2%	7,36	3,24 ± 1,39	44,02	6,50 ± 1,36	88,32	7,22 ± 1,55	98,02
Concentration 4%	7,31	1,86 ± 0,72	22,44	5,09 ± 1,49	72,78	6,65 ± 0,97	93,99
Positive Control	7,08	4,22 ± 1,23	59,60	6,78 ± 0,74	95,76	7,08 ± 0,38	100
Negative Control	7,07	2,85 ± 1,01	40,24	5,73 ± 0,95	81,84	6,78 ± 1,05	95,54

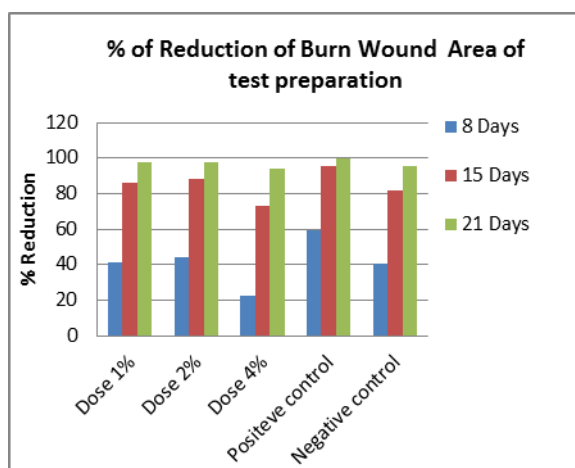


Figure 1. Percentage of Reduction of burn wound of gambir catechins dose 1%, dose 2%, dose 4%, positive control and negative control

The observations histopathology were done by using light microscope (Olympus SZ61) descriptively at 20 field of view with magnification 200 x and 400 x, as Shown in Figure 2.

Table 6. Results of Histopathology Assessment with Scoring System

Group	Score	
	Neocapillari zation	Infiltration of inflame matory cells
Positive Control	3	4
Negative Control	0	0
Consentration test 1%	1	2
Consentration test 2%	2	3
Consentration test 4%	0	1

Neocapillary formation or neovascularization is the formation of new blood vessels to the wound area. Inflammation of inflammatory cells is a process of inflammation of inflamed cells into the injury site. (Prasetyo, 2010). From the scoring results it can be observed that the highest neocapillarization score was found in the positive control group, followed by medium concentration test (2%), low concentration test (1%), high concentration test (4%) and negative control. The highest inflammatory cell infiltration score was in the negative control group, followed by high

concentration test (4%), medium concentration test (2%) and positive control.

From the results of microscopic observation, it was found the presence of inflammatory cells in the five groups. From the scores of inflammatory cell infiltration parameters it was found that 2% gambir catechins gel had the highest score after positive control compared to the other test group. High scores on inflammatory cell infiltration parameters signify at least inflammatory cells. This was because the catechins act as anti-inflammatory and antibacterial while control positive contains neomycin sulfate as an antibacterial so that the role of inflammatory cells to phagocytes microbes can be minimized and fast wound cleansing.

Table 7: Observation of Histopathology Scoring Reading based on Hosseini method, (2011).

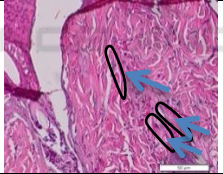
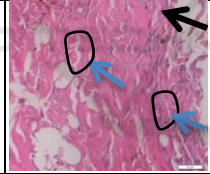
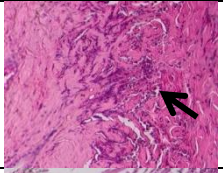
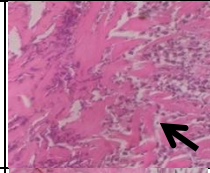
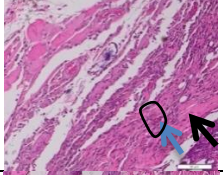
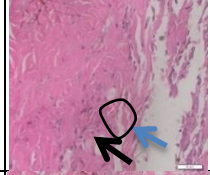

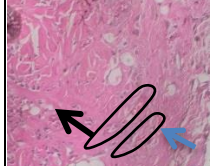
Score	Inflammatory cells	Angiogenesis
0	13-15 inflammatory cells per field of view	There is no angiogenesis, there is congestion, hemorrhagic and edema
1	10-13 inflammatory cells per field of view	1-2 blood vessels per field of view, there are edema, hemorrhagic, congestion
2	7-10 inflammatory cells per field of view	3-4 blood vessels per field of view, there are edema, hemorrhagic, congestion
3	4-7 inflammatory cells per field of view	5-6 blood vessels per field of view, there are edema, hemorrhagic, congestion
4	1-4 inflammatory cells per field of view	More than 7 blood vessels per field of view, there are edema, hemorrhagic, congestion

In contrast, the lowest scores were in the negative group and the high concentration test (4%). Low scoring of inflammatory cell infiltration parameters signifies the number of inflammatory cells. This was

caused by the absence of active ingredients in the preparation that could help to eliminate the foreign particles so it was possible that microbial and tissue damage must be phagocytized by the cells in the wound area so that the inflammation rate was still high. This suggests that inflammation and phagocytosis were still occurring and indicate that the two test groups were still in the inflammatory phase.

In the neocapillarization parameter, the highest scores were obtained in the medium concentration test (2%) after positive control compared to the other test groups. High scoring on neocapillarization parameters showed an increase in the number of neocapillaries that signaled the process of wound healing in the proliferative phase.

The important process of cellular activity in this phase is repair and heal wounds and was characterized by cell proliferation (Hosseini et al, 2011). Moreover, inflammatory cell infiltration scores in the moderate concentration test group (2%) had high scores. This shows that in the medium concentration test group (2%) has passed the inflammatory phase and entered the proliferation phase.

Group	Magnification	
	200x	400x
Positive Control		
Negative Control		
Concentration Test 1%		
Concentration Test 2%		

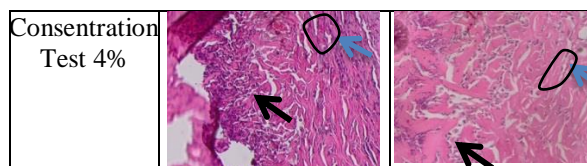


Figure 2: Result of Observation of Histopathology of white rats skin tissue with Hematoxylin-Eosin staining at 200x and 400x magnifications

Note:

Black arrow : Inflammatory cells

Blue arrow : Neocapillarization

From the analysis of all data, it can be concluded that the activity of gambir catechins in healing process of second degree burn did not show significant result on decreasing of burn area and percentage of wound healing. However, the activity of catechin isolates of gambir has affects the healing of burns in the inflammatory phase and proliferative phases. This was seen in histopathologic images. where in inflammatory cell, the medium concentration test group (2%) could decrease the number of inflammatory cells compared to the low concentration test group (1%), high concentration test (4%) and negative control. This was because gambir catechins have activity as anti-inflammatory and antibacterial. (Murdja et al 2017, Murdja et al 2018). The existence of these compounds would indirectly decrease the stimulation of inflammatory cell migration to the wound area so as to reduce the number of inflammatory cells and the process of clearance of foreign substances can run faster Hosseini et al, 2011). Thus, the moderate concentration test group (2%) may affect the healing of burns in the inflammatory phase.

In the neocapillarization parameter, the moderate concentration test group (2%) showed a higher score than the low concentration test group (1%), high concentration test (4%) and negative control. In the process of tissue repair, the presence of blood vessels had an important role to provide nutrient intake for tissue that was regenerating. In addition, blood vessels also had a role to deliver inflammatory cells formed in the marrow to close the wounded tissue so that the inflammatory cells to emigrate. To support these functions, blood vessels would form new vascular shoots that will eventually develop into a new branching on the wound tissue or called neocapillarization (Hananeh et al 2015). Thus, the number of new blood vessels in the injury site in the medium concentration test group (2%) indicates that

the wound healing process had proceeded in the proliferation phase. (Eva et al, 2014)

Anatomic and histopathology observations showed differences between groups. This difference explains that in each gel preparation group had a different work force. The working strength of the gel preparation was influenced by the active ingredient content of each gel and the concentration of isolates that affect its activity both in the inflammatory phase, the proliferation phase and the maturation phase. In general, the moderate concentration test group (2%) showed better results than the low concentration test group (1%) and the high concentration test group (4%). This is in line with the research conducted by Sumoza et al. (2014) where in the study of Gambir's Influence on Healing Burns on white mice showed good results in moderate concentrations.

In the medium concentration test group (2%) showed the best results microscopically followed by low concentration test group (1%) and high concentration test group (4%). However, in the high concentration test group (4%) showed unsatisfactory results. This was probably due to the low concentration of 1% gambir catechins gel having the active ingredient content below the optimum dose so that although it diffuses well but the active ingredient content was not sufficient for wound healing, medium concentration (2%) had the active ingredient content in the range optimal dosage for wound healing while gambir catechins gel with high concentration (4%) was above the optimal dose for wound healing. This causes the high concentration of 4% gambir catechins gel to have high viscosity and cause the gel to be too thick resulting in the release of the active substance and the penetration slowing into the skin.

4 CONCLUSION

1. Gambir Catechins (*Uncaria gambir*, Roxb) on the concentration group of 1%, 2%, 4%, control positive and control negative did not significantly different ($p > 0,05$) in reducing the surface area on second degree burn wound which was given topically on anatomical pathology observation.
2. There were differences in infiltration of inflammatory cells and neocapillarization on day 7 in the 2% concentration test compared with the 1% concentration test, 4% concentration test and negative control on microscopic observation, where

the medium concentration group (2%) was better than low concentration test group (1%) high concentration test group (4%) of gambir catechins and negative control.

3. Gambir catechins could assist in the healing process of burns in the inflammatory phase and proliferation.

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REFERENCES

- Akhoondinasab MR, Akhoondinasab M, Saberi M. 2014. Comparison of Healing Effect of Aloe Vera Extract and Silver Sulfadiazine in Burn Injuries in Experimental Rat Model. Original article Vol. 3 No. 1; 29-34
- Ambiyani, Winny., 2013, (In bahasa) Pemberian Salep Ekstrak Daun Mengkudu (*Morinda Citrifolia* L) Meningkatkan Proses Regenerasi Jaringan Luka Pada Tikus Putih Galur Wistar (*Rattus Norvegicus*) Jantan. (Administration of Noni Leaf Extract (*Morinda Citrifolia* L) Improves Wound Tissue Regeneration Process in Male Wistar (*Rattus Norvegicus*) Male White Rat, Thesis. Denpasar: Universitas Udayana
- Angraini T, Tai A, Yoshino T, Itani T, 2011. Antioxidative activity and catechin content of four kinds of *Uncaria gambir* extracts from West Sumatra Indonesia. African Journal of Biochemistry Research, 5(1), pp 33-38
- Ansel, H.C. 2005, (In bahasa), Pengantar Bentuk sediaan Farmasi (Introduction of Pharmaceutical Dosage form). Edisi 4. Penerjemah: Farida Ibrahim. Jakarta: UI Press.
- Arakawa H1, Maeda M, Okubo S, Shimamura T., 2004, Role of hydrogen peroxide in bactericidal action of catechin. Biol Pharm Bull. Mar;27(3):277-81.
- Atiyeh, B.S.; Costagliola, M.; Hayek, S.N., 2007, Effect of silver on burn wound infection control and healing: Review of the literature. Burns, 33, pp 139-148.
- Ditjen POM RI, (2000), Departemen of Health RI, General Standard Parameters of Medicinal Plant Extracts. (In bahasa) Parameter Standar Umum Ekstrak Tumbuhan Obat.
- Eva E, Noo C, Dina R. (2014), The Effect of Quercetin gels on the number of neutrophils and lymphocytes in the process on second grade burn wound healing in wistar male rats (In bahasa: Pengaruh pemberian gel kuersetin terhadap jumlah neutrofil dan limfosit dalam proses penyembuhan luka bakar derajat ii a

- pada tikus jantan galur wistar), *Journal Pharmascience*, Vol 1, No. 2, Oct, 38 - 45
- Farsworth, N.R., 1969, Biological and Phytochemical Screening of Plants, *Journal Pharmaceutical Science*. 55 (3): 255-276.
- Ferdinal N, A., 2014, Simple Purification Method of Catechin from Gambier, *International Journal on Advanced Science Engineering Information Technology*, Vol.4, No. 6, ISSN: 2088-5334
- Hananeh WM, Ismail ZB, Alshehabat MA, Ali J, (2015) Review of animal models used to study effects of bee products on wound healing: findings and applications. *Bull Vet Inst Pulawy* 59, 425-431.
- Harborne JB. *Phytochemical methods: A guide to modern techniques of plant analysis*. 3rd ed. London: Academic Press; 1998. p. 192-204.
- Handayani Fitri, Siswanto Eka dan Pangesti Lintang A.T., 2015, (In bahasa) Uji Aktivitas Ekstrak Etanol Gambir (*Uncaria gambir*) Terhadap Penyembuhan Luka Bakar Pada Kulit Punggung Mencit Putih Jantan (*Mus musculus L.*) . *Jurnal Ilmiah Manuntung I(2)*, 133-139
- Hargono, D, (1986), Galenic preparations, Directorate General of Drug and Food Control, Department of Health RI, (In bahasa) Sediaan Galenic, Direktorat Jendral Pengawasan Obat dan Makanan (BPOM), Departemen Kesehatan Republik Indonesia, Jakarta.
- Heitzman E.M., Neto C.C., Winiacz E., Vaisberg A.J., Hammond G.B., 2005, .Ethnobotany, phytochemistry and pharmacology of *Uncaria* (Rubiaceae). *Phytochemistry*, 66, 5 – 39.
- H. Niknejad, G. Paeni-Vayghan, F.A. Tehrani, M. Khayat-Khoei, H. Peirovi (2013), Side dependent effects of the human amnion on angiogenesis *J.Placenta* 34 340-345, [https://www.placentajournal.org/article/S0143-4004\(13\)00071-4/pdf](https://www.placentajournal.org/article/S0143-4004(13)00071-4/pdf).
- Isnawati, A., 2010, (In bahasa) Analisa Kualitatif dan Kuantitatif Senyawa Katekin dan Kuersetin pada 3 Mutu Ekstrak Gambir. Laporan Akhir. Program Insentif Riset Terapan : Teknologi Kesehatan dan Obat. Pusat Penelitian dan Pengembangan Biomedis dan Farmasi. Badan Litbang Kesehatan Departemen Kesehatan. <http://km.ristek.go.id/asset/files/479/pdf>. Diakses 1-11.2013
- Jose P. Sterling, MD, David M. Heimbach, MD, FACS, and Nicole S. Gibran, MD, FACS, (2010), Management of the burn wound, *Decker Intellectual Properties*, DOI 10.2310/7800.S07C15. <https://pdfs.semanticscholar.org/c0e1/05f2cf48de1720a749933a8b0bdc43bede50.pdf>.
- Kaddoura I, Abu-Sittah G, Ibrahim A, Karamanoukian R and Papazian N, (2017), Burn injury: review of pathophysiology and therapeutic modalities in major burns, *Journal Ann Burns Fire Disasters*, v.30(2); Jun 30, 95–102. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5627559/>.
- Kulac M1, Aktas C, Tulubas F, Uygur R, Kanter M, Erboga M, Ceber M, Topcu B, Ozen OA.(2013) *J Mol Histol.* (2013) The effects of topical treatment with curcumin on burn wound healing in rats. *J Mol Histol.* Feb;44(1):83-90. Doi:10.1007/s10735-012-9452-9. <https://www.ncbi.nlm.nih.gov/pubmed/23054142>.
- Li, J.; Zhao, G.Z.; Chen, H.H.; Wang, H.B.; Qin, S.; Zhu, W.Y.; Xu, L.H.; Jiang, C.L.; Li, W.J., 2013, Antitumour and antimicrobial activities of endophytic streptomycetes from pharmaceutical plants in rainforest. *Lett. Appl. Microbiol.* 47, 574–580.
- Lucida, H., Bakhtiar, A. dan Putri, W.A. 2007, (In bahasa) Formulasi sediaan antiseptik mulut dari katekin gambir (Formulation of oral antiseptic preparations from gambir catechins). *Jurnal Sains Tek. Farmasi.* 12(1), 7-14
- M. J. Hoekstra, P. Hupkens, R. P. Dutrieux, M. M. C. Bosch, T. A. Brans and R. W. Kreis, (1993), Comparative burn wound model in the New Yorkshire pig for the histopathological evaluation of local therapeutic regimens: silver sulfadiazine cream as a standard, *British Journal of Plastic Surgery*, 46, 585-589 <https://www.ncbi.nlm.nih.gov/pubmed/8252267>.
- M. Amini, M. Kherad, D. Mehrabani, N. Azarpira, M. R. Panjehshahin & N.Tanideh (2010) Effect of Plantago major on Burn Wound Healing in Rat, *Journal of Applied Animal, Research*, 37:1, 53-56, DOI: 10.1080/09712119.2010.9707093. To link to this article: <https://doi.org/10.1080/09712119.2010.9707093>.
- M. N. Shahzad, N. Ahmed, (2013), Effectiveness of Aloe Vera Gel compared with 1% silver sulphadiazine cream as burn wound dressing in second degree burns, *J Pak Med Assoc.* Vol. 63, No.2, February, 225 – 230. http://jpma.org.pk/full_article_text.php?article_id=4001.
- Moenadajat Y., 2009, (In bahasa) Luka bakar masalah dan tata laksana. Jakarta: Balai Penerbit FKUI.
- Mohamed El-Maghawry HAM, Nem WE, Sherif N-A, Hagag SA., 2008, An interventional study to decrease healthcare associated burn wound infections in the burn unit of Al Ahrar Hospital in Zagazig city, Sharkia Governorate. *International Journal of Current Microbiology and Applied Sciences* (2016) 5: 566-578. 57.
- Musdja MY, Hapsari MA and Agusta A, (2017), Comparison of Activity and Inhibitory Mechanism between (+)-Catechin and Water Extract of Gambier (*Uncaria Gambir* Roxb.) Against Some Bacteria, *Scientific Journal of PPI-UKM*, Vol. 4 (2017) No. 2, 55-60. DOI: 10.27512/sjppi-ukm/se/a29012018. <http://www.kemalapublisher.com/index.php/ppi-ukm/article/view/300>.
- Musdja MY, Rahman H A & Hasan D, (2018). Antioxidant Activity of Catechins Isolate of *Uncaria Gambier* Roxb in Male Rats. *LIFE: International Journal of Health and Life Sciences*, 4(2).34-46. DOI-<https://dx.doi.org/10.20319/ijlhls.2018.42.3446>. <http://www.grdspublishing.org/index.php/life/article/view/1388/1172>.

- National Standard Indonesia: SNI 01-3391-2000, http://infopk.bsn.go.id/index.php?/sni_main/sni/detail_sni/3793.
- Ningsih Sri et al., 2014, Evaluation Of Antilipid Peroxidation Activity Of Gambir Extract On Liver Homogenat In Vitro. International Journal of PharmTech Research. ISSN : 0974-4304 Vol.6, No.3, 982-989
- Pakki, Dkk., 2009, (In bahasa), Uji Aktivitas Antibakteri Enzim Papain Dalam Sediaan Krim Terhadap *Staphylococcus Aureus*. Majalah Farmasi Dan Farmakologi Vol.13, No.1 (Issn: 1410-7031).
- Pambayun et al., 2007, Phenolic Content of Gambir Leaf Extract and Antibacterial Activity, (In bahasa) Kandungan Fenolik Ekstrak Daun Gambir Dan Aktivitas Antibakterinya. AGRITECH vol. 27 No. 2
- Prasetyo, B.F., Wientarsih, I., Priosoeryanto, B.P., 2010, Activity of Ambon Banana Tree Stem Extract Gel in the Process of Healing Wounds in Mice, (In bahasa) Aktivitas Sediaan Gel Ekstrak Batang Pohon Pisang Ambon dalam Proses Penyembuhan Luka pada Mencit. Jurnal Veteriner. Vol: 11, No 2 : 70-73
- Rowan, M.P.; Cancio, L.C.; Burmeister, D.M.; Rose, L.F.; Natesan, S.; Chan, R.K.; Christy, R.J.; Chung, K.K., 2015, Burn wound healing and treatment: Review and advancements. Crit. Care 19, 243, doi:10.1186/s13054-015-0961-2.
- Rini A, Oktadefitri Y, Lucida H., 2013, Sunscreen cream formulation of ethyl p-methoxycinamate combination with catechins, Proceedings of the National Seminar on the Latest Development of Pharmaceutical Sciences and Clinics III (In bahasa : Formulasi krim tabir surya dari kombinasi etil p – metoksisinamat dengan katekin), . Prosiding Seminar Nasional Perkembangan Terkini Sains Farmasi dan Klinik III ISSN: 2339-2592
- Tehrani S, Lotfi P, Tehrani S, Jangholi E, Aryan H, Aidun A, (2016) Healing Effect of Sesame Ointment on Second degree Burn Wound in Rats, GMJ, 5(2):56-62, www.gmj.ir, file:///C:/Users/User/Downloads/658-3565-5-PB.pdf.
- Shahzad MN and Ahmed N (2013), Effectiveness of Aloe Vera Gel compared with 1% silver sulphadiazine cream as burn wound dressing in second degree burns, J Pak Med Assoc, Vol. 63, No.2, February, 225-230
- Sjamsuhidayat R, Wim de Jong., 1997, (In bahasa) Buku Ajar Ilmu Bedah. Edisi 2. EGC, Jakarta.
- S.V. Hosseini, H. Niknahad, N.Fakhar, A. Rezaianzadeh and D. Mehrabani, (2011), The Healing Effect of Honey, Putty, Vitriol and Olive Oil in *Pseudomonas Aeroginosa* Infected Burns in Experimental Rat. Model. Asian Journal of Animal and Veterinary Advances 6 (6), 572-579 ISSN 1683-9919 / DOI: 10.3923/ajava, 572.579.
- Somboonwong J, Kankaisre M, , Boonyong Tantisira B and Tantisira MH, (2012), Wound healing activities of different extracts of *Centella asiatica* in incision and burn wound models: an experimental animal study, BMC Complementary and Alternative Medicine 2012 12:103. doi:10.1186/1472-6882-12-103. <http://www.biomedcentral.com/1472-6882/12/103>.
- Standar Nasional Indonesia (National Standard of Indonesia : SNI 01-3391-2000. http://teknologihutan.fkt.ugm.ac.id/userfiles/download/SNI_01-3391-2000_-_Gambir.pdf
- Sumoza Nelsy, Sucidayana, Efrizal, Rahayu Resti., 2014, (In bahasa) Pengaruh Gambir (*Uncaria gambir* R.) Terhadap Penyembuhan Luka Bakar Pada Mencit Putih (*Mus musculus L.*) Jantan [Effect of Gambir (*Uncaria gambir* R.) on Healing Burns in Male White Mice (*Mus musculus L.*)], Jurnal Biologi Universitas Andalas 283-288 (ISSN : 2303-2162)
- Taniguchi S., Kuroda K., Yoshikado N., Doi K.I., Tanabe M., Shibata T., Yoshida T., Hatano T., 2008, New Dimeric Flavans From Gambir, an Extract of *Uncaria gambir*. Japan: The Japan Institute of Heterocyclic Chemistry, Okayama University, 1-11. <http://ousar.lib.okayama.ac.jp/files/public/3/34205/20160528031815252292/fulltext.pdf>.
- Voravuthikunchai, S.P. and Mitchell, H., 2008, Inhibitory and killing activities of medicinal plants against multiple antibiotic-resistant *Helicobacter pylori*. J Health Sci 54, 1–8.
- W. Hananeh, Ismail ZB, Alshehabat MA, Ali J, (2015), Review of animal models used to study effects of bee products on wound healing: findings and applications, Bull Vet Inst Pulawy 59, 425-431, 2015, DOI:10.1515/bvip-2015-0062. <https://www.degruyter.com/downloadpdf/j/bvip.2015.59.issue-3/bvip-2015-0062/bvip-2015-0062.pdf>.
- Wasserman, D., 2002, Criteria for burn severity: Epidemiology, prevention, organization of management. Pathol. Biol. 50, pp 65–73