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Research Article

Effect of Gel Formulation of Corn Silk Extract (*Stigma Maydis*) On Burn Wound Healing In Male White Rat

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ABSTRACT

Objectives: Burns are damage or loss of tissue caused by exposure to very high temperatures. This study was to determine the effect of gel formulation from corn silk (*Stigma maydis*) in healing burns in male white rats.

Data Sources Study Selection: This research is an experimental research of the effect of gel formulation from corn silk (*Stigma maydis*) in healing burns in male white rats

Summary of contents of the article: Five groups are used in this study. Group I (induced hot metal), group II (induced hot metal + gel base), group III (induced hot metal + 10% corn silk extract gel), group IV (induced hot metal + 15% gel of corn silk extract gel), group V (induced hot metal + B® gel). Observations were made for 21 days. The results of this study are the average % of burns healing on day 7, 14 and 21 : group I (27.29%, 63.03%, 100%), group II (35.39%, 60.16 %, 100%), group III (37.16%, 65.34%, 100%), group IV (36.89%, 68.23%, 100%), group V (32.81%, 66, 48%, 100%). The period of epithelialization of skin tissue are groups I, II, III on day 16, group IV on day 14 and group V on day 17. The mean results of the epithelialization scores for groups I, II, III, IV and V (2, 2, 3, 3, 3), the mean fibroblast score results (2,7, 2,9, 3, 3, 3), groups I, II, III, IV and V collagen fiber density groups I, II, III , IV and V (2,2, 2,8, 3,6, 3,9, 3,9). The two-way ANOVA statistical analysis test on % burn healing was not significant (p> 0.05), and the one-way ANOVA statistical analysis test at the period of epithelialization was not significant (p>0.05) and the histology of epithelialization and fibroblasts was not significant (p>0.05), the density of collagen fibers was significant (p<0.05).

Conclusion: The conclusion of this study is corn silk extract gel (*Stigma maydis*) 10% and 15% have the effect on the healing process of burns and can be formulated in gel dosage forms.

Keywords: Burn; Corn Silk Extract (*Stigma maydis*); Epithelialization Period; Histopathology.

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INTRODUCTION

Burns (Combustio) is a form of tissue damage or loss caused by contact with a source that has a very high temperature (for example fire, hot water, chemicals, electricity and radiation).[1] Burns are one of the injuries that can affect anyone. It is estimated that one in about 3.5 million people will experience burns.[2] World Health Organization (WHO) estimates that there are 265,000 deaths that occur annually world-wide due to burns.[3] In Indonesia, the prevalence of burns in 2013 was 0.7% and has decreased by 1.5% compared to 2008 (2.2%).[4]

Although the management to burns has developed rapidly and the prevalence has decreased each year, the risk of infection through skin cuts or openings is still high. [5] The action that can be done in burns is to provide local therapy with the aim of getting healing as quickly as possible.[6] Based on this, several studies have begun to be developed for the treatment of burns from natural ingredients, one of which is corn silk (*Sigma maydis*).Another research results

tested the effectiveness of corn silk in cream formulation (*Zea mays* L.) to healing wound mice diabetes mellitus.[7]

Corn silk knowed has chemical components that can be used and support the healing process of burns, this component is corn silk (*Stigma maydis*) contain anthraquinones, alkaloids, flavonoids, glycosides, saponins, steroids, tannins, terpenoids, andphenols.[8] Flavonoids has activity as an antioxidants on that it can accelerate the inflammatory phase and prevent cell damage and cellular components caused by free radicals.[9] Saponins area compound capable of spurring the formation of collagen, a structural protein that plays a role in the process healing wound all at once has the ability to be an effective cleanser to heal open wounds.[10]

One of the pharmaceutical preparations that are often used for healing burns is gel. This dosage form is easier to use and spreads over the skin faster. In addition, the gel has properties that are soothing, moisturizing, easy to penetrate the skin so provide healing effect. Gel preparations can protect the skin from excessive dehydration. Formulation and selection of the right base in the manufacture of gel preparations will affect the amount and rate of active substances that will be absorbed. Ideally, the base and carrier should be easy to apply to the skin, non-irritating and comfortable to use on the skin.[11] Gel has several advantages such as easy use, attractive shape, easy to wash with water, stable preparation, and is a good conductor, for drugs used on skin tissue or mucous membranes.[12]

METHODS

Material

Corn silk, 70% Ethanol, Aquades, Chloroform, FeCl3, Mg Powder, Norit, Anhydrous Acetic Acid, Acetic Acid, Phosphate Buffer, H2SO4 2N, H2SO4 (p), HCI (p), Mayer, Methyl Parabens, Carbopol 940, TEA, Glycerin, Propylengicol, Propyl Paraben, Aqua Distillation, Alcohol 70%, 80%, 90%, 96% 100%, Formalin 10%, Xylol, Paraffin, Haematoxylin-eosin, Canadian Balm / Entellan, and gel B®.

Experimental animals

Male white rats aged 2-3 months with a weight ranging from 250-300 grams.

Corn Silk Extract Preparation (*Stigma maydis*)

4 kg of corn silk (*Stigma maydis*) was cleaned and dried and chopped into small pieces and aerated with 70% ethanol solvent, for 4 days. Meserate is filtered and then concentrated with a rotary evaporator to obtain a thick extract.

Phytochemical Evaluation of Corn Silk Extract (*Stigma maydis*)

The thick extract of corn silk was weighed 0.5 grams and then put into a test tube. Add chloroform and water each 5 ml (1: 1) then shake vigorously and let it for a while until 2 layers are formed, namely water and chloroform.[13]

Flavonoid Test

1–2 drops of a layer of water are placed on a drop plate, add a little Mg metal powder and a few drops of HCl (p), the appearance of a yellow-orange to red color indicates the presence of flavonoids.

Tannin Test

1-2 drops of a layer of water are placed on the drop plate, then add 1-2 drops of 31% FeCl reagent, the formation of green, purple, blue or dark black color indicates the presence of tannins.

Saponin Test

The water layer is put in the test tube then shake it, if a permanent foam is formed (± 15 minutes) it indicates the presence of saponins.

Alkaloid Test

The alkaloid test is carried out with 2-3 drops of chlorophore layer added with 10 ml of ammonia chlorophore and 1 drop of 2 N sulfuric acid, then shake vigorously and let stand until two layers are formed, take the acid layer then add 1-2 drops of major reagent, the positive reaction of the alkaloid is marked in the presence of a white mist to a white mass.

Gel Formulation of Corn Silk Extract Preparation

Carbopol 940 10% is infused with hot water while stirring occasionally until Carbopol 940 expands to form a thick mass in the mortar then TEA 3% is added and crushed until a clear gel mass is obtained and 5% glycerin (M1) is added. 0.1% propyl paraben and 0.2% methyl paraben were dissolved in ad soluble 5% propylene glycol (M2). Gradually put the corn silk extract into M1, crush it until it is homogeneous, M2 is added and then crushed until a homogeneous preparation is formed, then put it in a container.

Evaluation of Corn Silk Extract Gel

Organoleptic Examination

Observations of shape, smell and color were carried out visually, left to room temperature and observed every week for 6 weeks.[14]

Homogenity Examination

The gel is weighed 0.1 g, then placed on a glass slide then scratched with a glass cover to form a flat surface then covered with a glass cover and note whether there is any inhomogeneity under the light. Preparations must show a homogeneous arrangement and do not show coarse grains and be observed every week for 6 weeks.[15]

PH

This examination is carried out using a pH meter. This tool is calibrated first using an acetate buffer pH 4.0 and a phosphate buffer pH 7.0 so that the needle position indicates the pH value. The electrodes were rinsed with distilled water and dried. The examination is carried out by measuring the gel preparation by dipping the electrodes into the container. Let the numbers move at a constant position. The number shown by the pH meter is the pH value of the preparation and is observed every week for 6 weeks.[15]

Stability

The stability test using the Cycling Test method was carried out to see the stability of a preparation with the influence of temperature variations during a certain storage time. The preparation was stored at cold temperature (4 ± 2 ° C) for 24 hours, then transferred to an oven at 40 ± 2 ° C for 24 hours. This treatment is called 1 cycle. The test was carried out in 6 cycles and observed physical.[16]

Viscosity

The tool used is a stormer viscometer. The gel preparation was put into a 250 mL beaker. Measurements are made by dipping the spindle into the gel preparation up to the boundary markings on the spindle, then turning on the tool. Examinations were carried out in the first week and the sixth week.[17] Good gel viscosity values are 2000-4000 cPs.[18]

Study Design

Animals are weighed and grouped into 5 groups, each group consisting of 5 animals. Group I Mice induced with hot metal. Group II Rats induced with hot metal and smeared with 0.2 gram gel base preparation. Group III Rats induced with hot metal and smeared with a 10% concentration of 0.2 grams of extract gel. Group IV Rats induced with hot metal and smeared with a 15% concentration of 0.2 grams of extract gel. Group V Rats induced with hot metal and smeared with a circulating preparation, namely 0.2 gram B® gel. Apply 2 times a day.

Parameters Measured In Wound Healing

Percentage of burn wound healing

The percentage of burns healing was calculated on day 7,14 and 21 with the formula:

% Burn Wound Healing =

$$\frac{\text{Initial burn size} - \text{spesific day burn size}}{\text{The area of the initial burn}} x\ 100\ \%$$

Period of Epithelialization

The time required for the formation of a complete new epitheliumon burns. In this case it is recorded on what day the scab tissue is peeling from the wound without leaving any residue.

Histological Preparations

Observations were made on the burn tissue. From each group, 3 rats were taken to be decapitated each on day 21. Samples of burn tissue were taken 0.3 cm from the edge of the burn, the burn tissue fixation was immersed in 10% formalin solution for 1- 4 days (tissue wet). Perform tissue processing, Hematokxylin-eosin staining and microscopic examination of burn tissue histological preparations.

Statistical Analysis

Data from the test results of the gel containing corn silk extract (*Stigma maydis*) with a concentration of 10% on the healing of burns in male white rats with parameters of% burn healing, epithelialization time and collagen fiber density were statistically processed by one-way ANOVA and two-way ANOVA using SPSS 23 and continued with Duncan's test. Two-way ANOVA statistical test for % of burn healing parameters, and one-way ANOVA statistical test for parameters of epithelialization time and collagen fiber density.

DISCUSSION

Table: 1 Results of Corn Silk Extract Phytochemical Screening Test

Chamical content	Observation result	Conclusion
Alkaloids	No white / white precipitate	-
Flavonoids	Yellow to orange	+
Saponins	Foamy (hold 15 minute)	+
Tannins	Green, purple, blue or deep green	+

Table: 2 Gel Concentration Formulations

Composition	F0	F1	F2
Ekstrak silk corn (%)	-	10	15
Carbopol 940 (%)	1	1	1
TEA (%)	q.s	q.s	q.s
Glycerin (%)	5	5	5
Propylene glykol (%)	5	5	5
Methyl paraben (%)	0,2	0,2	0,2
Propyl paraben (%)	0,1	0,1	0,1
Aqua destilata sampai (%)	100	100	100

F0 = Gel base
F1 = Corn silk extract gel formula 10%
F2 = Corn silk extract gel formula 15%

Table:3. Results of Evaluation of Gel Preparations

No	Evaluation			Observation	
		F0	**F1**	**F2**	**B® gel**
1.	Organoleptic -shape -color -smell	 SP T TB	 SP CM BE	 SP C BE	 SP T TB
2.	Homogeneity	H	H	H	H
3.	pH	7,07	6,73	6,45	6,86
4.	Viscosity test	3194 cPs	3953 cPs	2215 cPs	2353 cPs
5.	Stability test	TM	TM	TM	TM

Information :

F0: Gel base
F1: Gel 10% concentration
F2: Gel 15% concentration
TB:Odorless
T: Transparent
H: Homogeneous
CM: Light Brown
TM:NotSeparating
C: Brown
SP: Semi-Solid
BE: Extract odor

Corn silk extract was obtained by maceration method using 70% ethanol as solvent. Maceration was chosen because it is good for compounds that are not resistant to heat and has several advantages including simple equipment used and easy processing.[14]

The macerated filtrate is evaporated using a vacuumrotary evaporator in order to remove the solvent so that a thick extract is obtained.

Standardization of non-specific parameters carried out in this study were the ash content test, dryer shrinkage test, yield and phytochemical test. For susceptibility to the requirements for the ash content test, drying shrinkage, and yield in the 2017 Indonesian herbal pharmacopoeia for a yield of not less than 3.8% , in the dryer shrinkage test is not more than 11% and the ash content is not more than 5%. In the results obtained for the non-parametric test yield, drying shrinkage and ash content all the tests met the requirements.

Furthermore, the extract is made into a gel dosage form. After the preparation was formulated, the preparation was then evaluated organoleptically, homogeneity, viscosity, and pH. Each test was carried out for 6 weeks.For the organoleptic test of the preparation From the organoleptic observations of the gel, it was obtained that a clear gel base preparation was a characteristic of carbopol 940 which gave a transparent effect to the gel, odorless, with a semi-solid consistency. The 15% concentration gel has a dark brown color, which is the color of the corn silk extract with the extract's distinctive aroma with a semi-solid consistency. The lower the concentration of corn silk extract, the faded the brown color of the 10% concentrated corn silk extract gel. In the evaluation of the homogeneity of corn silk extract gel, the concentration of 10% and 15% was the result that the gel was well mixed or homogeneous. In the pH test for a good pH gel requirement, the pH ranges from 4.5 to 7.5 which means that the pH of the corn silk extract gel with a concentration of 10% and 15% meets the criteria for a good gel. In the viscosity test, a good gel viscosity value is in the range 2000-4000 cPs, because with this viscosity the gel is able to spread well when applied. From the results of the viscosity test, the corn silk extract gel met the requirements for good gel viscosity.

The cycling test was conducted to determine whether the preparation had undergone phase separation after being stored at two different temperatures, namely 4ºC and 40ºC. Observation of corn silk extract gel using the cycling test method was carried out for 6 for 12 days, in which one cycle was carried out for 48 hours, the gel was put in an oven for 24 hours at room temperature (40ºC) and 24 hours at cold temperature (4ºC).[16] Based on the observations, it can be seen that all formulas do not change in physical appearance either from shape, smell, color, and do not experience separation at either 4 ° C or 40 ° C. This shows that all the ingredients used are able to mix well and the preparation is stable both in low and high temperature storage. The preparation is considered good because it is stable for 6 cycles of testing.

Percentage of Burns Healing Area

The percentage of wound healing that was observed was the initial wound area measurement with the wound area measurement on days 7,14 and 21, where a high percentage indicated effective wound healing with the decreasing size of the wound from day to day. In the observations made, the wound began to shrink on day 3 to day 5 because it had undergone a hemostatic reaction, where the platelets that came out of the blood vessels were attached to each other accompanied by the formation of a scab. The speed at which scabs form from each treatment group indicates the speed of wound healing.[20] Meanwhile, on the 7th to 21st day the wound reduced faster. This indicates that the preparation has a better effect on the proliferative phase than the inflammatory phase.

From the measurement results of the percentage of burns healing on the 7th day, the average percentage of burns healing area in the hot metal induction group group: 27.92% ± 8.62, the 10% gel extract group: 37.16 ± 8.38, gel group extract concentration of 15%: 36.89% ± 4.30, gel base: 35.39% ± 9.72, then B® gel: 32.81% ± 3.45, where the treatment group was smeared with a silk extract gel preparation. corn with a concentration of 10% gave the highest average percentage of wound healing compared to

all groups. Then on the 14th day, the average percentage of burns in the hot metal induction group was obtained: 62.42% ± 18.09, 10% concentration of extract gel: 65.34 ± 5.32, 15% concentration of extract gel: 68 , 23% ± 15.83, gel base: 60.16% ± 12.45, then B® gel: 66.48% ± 12.28, on day 14 it was seen that the 15% concentration group gave an average percentage of wound healing bigger than other groups. This may be due to differences in test preparations that can affect the speed of wound healing of each group, so that different results are obtained for each group of test animal treatment. Then on day 21 it was found that all the experimental animal treatment groups were declared to be healed without leaving scars (inflammatory cells) because the healing parameters of all mice were 100%.

Table: 5 Results of Percentage of Burns Healing Area

Grup	Mean ± SD % Wound Area on Day		
	Day 7	Day 14	Day 21
Hot Metal Induction	27.92 ± 9,64	62,42 ± 20,23	100 ± 0
Gel Base	35.39 ± 10,87	60.16 ± 13,94	100 ± 0
10% Extract Gel	37,16±8,38	65,34±5,32	100 ± 0
50% Extract Gel	36,89% ± 4,30	68,23% ±15,83	100 ± 0
B® gel	32.81 ± 3.86	66.48 ± 13,73	100 ± 0

Based on the results of statistical analysis with the two-way ANOVA test, the value was obtained (p> 0.05), meaning that the value was not significant, it can be concluded that there was no significant difference, there was no difference in effect on burn healing because the average percentage value of burn healing not much different.

Period of Epithelialization

Period of epithelialization is the time recorded from the first day of scab removal without leaving a scar. From the results of observations carried out for 21 days in experimental animals, the treatment group of gel preparations with a concentration of 15% peeling occurred on the 14th day, the group of gel preparations with 10% concentration, base gel and hot metal induction on average peeling of tissue occurred on day 16. and in the B® gel group the mean tissue peeling occurred on day 17In control of metal induction, there was accelerated epithelialization of the B® gel, this was due to the induction of the induction scab (scab) which was forced to peel so that it still left a scar (inflammation) on the skin of the rats.

This is evidenced when the scab begins to form and when the scab comes off. The discoloration of second degree burns occurs as the wound dries up. The scab removal time indicates that new cells have grown on the skin, which helps accelerate the removal of the scab and the edges of the wound are closer together. The scab is removed because the underlying tissue is dry. and the edges of the wound begin to be drawn to the center.[20] And from the test results, it was found that different epithelialization time results could be caused by differences in the concentration of the test preparation which could accelerate the growth of new epithelium, so that scab release could occur in different days.

Based on the results of statistical analysis with the one-way Anova test, the significance value was p <0.05, meaning that the value was significant, it could be concluded that there was or was a significant difference between the 15% group and the other groups.

Histopathology

Histopathological test carried out was an observation of collagen fibers and epithelialization of skin tissue that had grown back on the 21st day, from each group 3 rats were taken for decapitation. and analyzed.

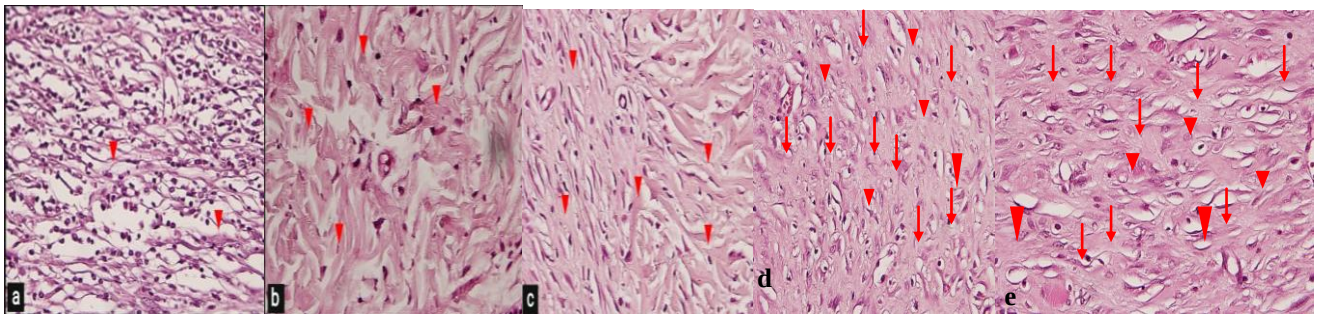

Figure: 1.Histopathological Observation Results, (a.) Hot Metal Induction, (b.) Gel Base, (c.) 10% Concentration Extract Gel, (d.) 15% Concentration Extract.

Note: Arrowheads (collagen) Darts (fibroblast cells)

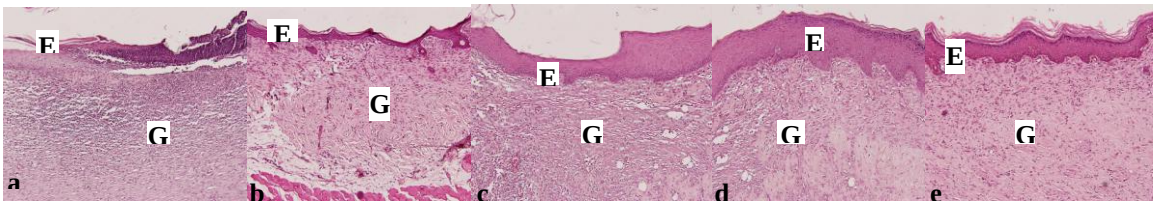

Figure: 2.Histopathological Observation Results, (a.) Hot Metal Induction, (b.) Gel Base, (c.) 10% Concentration Extract Gel, (d.) 15% Concentration Extract, (e.) B® gel.

Note: E = Epithelium G = Granulation tissue

Table: 6. Results of the histopathological score of collagen fibers

Group	Score	Results
Hot metal induction	2	Collagen fibers appear thin or slightly spread.
Gel base	3	Collagen fibers appear moderately dispersed or appear to be coalesced.
Gel of Corn silk extract 10%	4	Collagen fibers are spread out a lot and are completely bound
Gel of Corn silk extract 15%	4	Collagen fibers are spread out a lot and are completely bound
B® gel	4	Collagen fibers are spread out a lot and are completely bound

Table: 7 Results of the histopathological skin tissue epithelialization

Group	Score	Results
Hot metal induction	2	Incomplete
Gel base	2	Incomplete
Corn silk extract gel 10%	3	Complete
Corn silk extract gel 15%	3	Complete

In Figure 1. the density of collagen and fibroblast cells shows the granulation tissue in the post-burn dermis with fibroblast cells (arrows) and collagen matrix (arrowheads). Comparison of skin histology after burns in experimental burn induction group (a), positive control with gel base (b), treatment with 10% concentrated *Stigma maydis* extract gel (c), treatment with 15% concentrated *Stigma maydis* extract (d) treatment with B® gel (e). Shows granulation tissue in the post-burn dermis with fibroblast cells (arrows) and collagen matrix (arrowheads). In the induction control group, there was a loose granulation tissue with many inflammatory cells. In the treatment group with stigma extract gel, the fibroblast cells were more accompanied by denser collagen compared to the control group. In induction control animals most of the dermis contains granulation tissue with many inflammatory cells, but the density of collagen and fibroblasts is mostly still low. The administration of *Stigma maydis* extract showed a higher density of collagen and fibroblasts compared to hot metal induction and gel base, collagen density at 10% and 15% concentrations seemed to be equivalent to the density of collagen and fibroblast cells on comparison gel administration. An increase in the number of fibroblasts indicates an stimulating effect of collagen synthesis and fibroblast proliferation, while the reduction in inflammatory cells indicates an anti-inflammatory effect of Corn Silk extract (*Stigma maydis*) at a concentration of 15%.

From Figure 2 above shows the epithelial surface of the epidermis (E) and granulation tissue in the dermis (D) after burns, as well as crusts (scab) that cover the burn scars, on induction control there is a wound with a scab on the surface, the epithelium only covers part of the area , as well as loose granulation tissue with lots of inflammatory cells. In induction control, there was a wound with a scab on the surface, the epithelium only partially covered the area, as well as loose granulation tissue with many inflammatory cells. in the treatment with *Stigma Maydis* concentrations of 10% and 15% showed better epithelialization compared to the induction control and gel base, with thicker epithelium and denser connective tissue underneath. epithelial thickness at 15% concentration was slightly thicker than the B® gel.

Based on the results of statistical analysis on the collagen and epithelialization scores with the one-way Anova test, the sig.p value <0.05 indicated that the data obtained were different. Obtained sig value. of 0.00, which means that the sig.p value <0.05, so it can be concluded that there is a significant difference in collagen density between treatment groups. Whereas for the ANOVA test on the epithesis of the sig. p> 0.05, which means that there is no significant difference between treatment groups.

From the results of the hispatological test, it can be seen that the treatment with 15% concentration of corn silk extract gel (*Stigma maydis*) gives the impression that there is a faster improvement in burn healing in experimental animals which can be seen in the wound histology image of the experimental sample.

This result relates to the existence of secondary metabolites of corn silk plant which have medicinal properties. Secondary metabolites in corn silk include flavonoids, saponins and tannins[20]. Saponins can increase membrane permeability which causes cell hemolysis, if saponins interact with bacterial cells, the bacteria will lysis. Monocyte proliferation is enhanced by saponins which can increase the number of macrophages and secrete growth factors to produce fibroblasts and synthesize collagen to the

wound area. In addition, saponins can also accelerate the keratinocyte migration process which plays an important role in the re-epitalization process.[21] Other ingredients found in corn silk are flavonoids, tannins. Flavonoids have the ability as antioxidants that can reduce or change free radicals. Free radicals can inhibit cell proliferation, inhibit inflammatory reactions, and inhibit the contraction of the collagen tissue that is formed, all of which can inhibit the wound healing process.[22] Antioxidants will bind to free radicals which can cause damage to cell membranes so that cells cannot function properly. With this bond, the damage to the cell membrane can be reduced so that the proliferation phase can occur.[21] The flavonoid content in corn silk can function as a microbial destroyer, especially from the gram-negative bacteria. The mechanism of action of flavonoids functions as antibacterial by forming complex compounds against extracellular and dissolved proteins so that they can damage bacterial cell membranes and are followed by the release of intracellular compounds.[23] The tannins in corn silk act as an astringent which can cause the skin pores to shrink, stop light exudates and bleeding so as to cover the wound and prevent the usual bleeding in wounds. Tannins and saponins play a role in the migration and proliferation of fibroblasts in wounds so that wound contractions will be faster.[6]

CONCLUSION

From the results of the research the effect of corn silk extract gel with a concentration of 10% and 15% on the healing of burns in male white rats can be drawn the following conclusions: Corn silk (*Stigma maydis*) has activity in the healing process of burns and also Corn silk extract (*Stigma maydis*) with a concentration of 10% and 15% can be formulated into gel preparations.

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