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## THE EFFECTIVENESS OF MORINGA (*Moringa oleifera*) ROOT EXTRACT GEL IN HEALING INCISION AND DIABETIC WOUND

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*Skin is the outermost organ of the body that covers and protects the entire surface of the body. Incision wounds caused by a sharp object being cut and diabetic wounds caused by complications of diabetes. This study uses moringa (*Moringa oleifera*) root extract as the active substance because it acts as an antimicrobial that can accelerate wound closure and as an anti-inflammatory that can minimize the onset of inflammation. Moringa root extract is formulated into a gel because it is more effective in wound closure and easily release form the dosage forms. This study aims to determine the effectiveness of moringa root extract gel in healing incision and diabetic wounds. Moringa root extract gel effectiveness test was conducted on 6 treatment groups, namely K1: positive control (bioplacenton® gel and star ag® gel), K2: normal control (no treatment), K3: negative control (base gel), K4: 5% gel treatment, K5: 10% gel treatment and K6: 15% gel treatment. Data analysis using SPSS software with One Way ANOVA test. The results of the effectiveness test showed that moringa root extract gel with a concentration variation of 5%, 10% and 15% had a healing effect on incision and diabetic wounds. The results of the One Way Anova test show that the data on the percentage of healing of incision and diabetic wounds are different with a significant value of  $p < 0.05$  in each wound.*

**Keyword:** diabetic wound, extract, gel, incision wound, moringa root

### INTRODUCTION

Skin is one of the outermost and largest organs of the body that covers the entire surface of the body.<sup>1</sup> The skin plays an important role in protecting the body as a whole from various forms of harmful stimuli, such as physical and chemical trauma, microorganisms, and radiation.<sup>2</sup> The contact of the human body with the environment both consciously and unconsciously can cause the skin structure to be damaged and cause a condition called a wound. Wound is a condition of discontinuity of tissue due to injury or surgery which is characterized by the loss or damage of part of the body tissue which will interfere with the function of skin

tissue.<sup>3</sup> There are several kinds of wounds, including incision wound and diabetic wounds. Incision wound is a type of wound that results from the cutting of a sharp object, such as a knife. These wounds are most common in everyday life.<sup>3</sup> Diabetic wounds or diabetic ulcers are wounds caused by complications of diabetes. These wounds can develop into chronic, hard-to-heal wounds due to damaged blood vessels and bacterial infection.<sup>4</sup>

The wound healing process is generally divided into three stages, namely: inflammatory phase, proliferation phase and remodeling phase.<sup>5</sup> Wound care performed with modern dressings using the principle of moisture balance can accelerate the healing process by

always paying attention to the level of moisture around the wound.<sup>6</sup> Pharmacological treatment can be done in two ways, namely with synthetic and herbal medicine. Currently, many people prefer traditional or herbal medicine by using plants as a treatment.<sup>7</sup> One of the plants that can be used to treat wounds is Moringa, which is taken from the roots. Moringa (*Moringa oleifera*) root has activities as antimicrobial, anti-inflammatory, antidiabetic, wound healing.<sup>8</sup> Moringa root contains the secondary metabolite *pterygospermin* as an antimicrobial in accelerating the wound closure process<sup>9</sup> and *moringinine* alkaloid compound as anti-inflammatory.<sup>10</sup>

Moringa root extract is formulated into gel form because it is effective in drug delivery system.<sup>11</sup> The gel uses a hydrogel system that is able to absorb large amounts of water, this system acts as a wound dressing.<sup>12</sup> Wound dressings are capable of optimally removing various obstacles in the wound healing process.<sup>13</sup> The advantages of gel preparations are good drug distribution and release on the skin,<sup>14</sup> and easily absorbed into the skin.<sup>15</sup> The transparent, non-sticky gel is easy to wash off with water and provides a cooling effect on the skin surface, increasing comfort.<sup>11</sup>

Currently, there are many patients with diabetic ulcers or diabetic wounds<sup>16</sup> and incision wound (these wounds can occur consciously or unconsciously).<sup>17</sup> This study uses moringa plants because it refers to previous research showing that the administration of moringa leaf extract at a concentration of 100% can inhibit the growth of *Staphylococcus aureus* bacteria in diabetes mellitus wounds.<sup>18</sup> Other study reported the application of moringa leaf extract ointment at a concentration of 10% can heal incision wounds.<sup>19</sup> Previous investigation reported the variation of antimicrobial, antidiabetic, and antioxidant properties between the root and the leaf of *Moringa oleifera*<sup>20</sup>. However, there was limited study reporting the effectiveness of *Moringa oleifera* root extract gel in healing incisions and diabetic wound properties. Hence, this study was carried out to provide scientific information regarding this issue.

This study was conducted with the aim to determine the effectiveness of moringa root extract gel with different concentrations in

healing incision and diabetic wounds. The results of this study are expected that Moringa (*Moringa oleifera*) root extract gel can provide optimal results in healing incision and diabetic wounds and be an alternative treatment from natural materials that are locally available to the community.

## MATERIALS AND METHODS

### Materials

Moringa root, 96% ethanol (technical grade), carbopol 940 (USP grade), triethanolamine (TEA) (USP grade), propylene glycol (USP grade), methyl paraben (USP grade), glycerin (USP grade), distilled water reagent grade, Streptozotocin (STZ) (Merck KGaA, pharmaceutical grade), ketamine (Merck KGaA, pharmaceutical grade), bioplacenton® gel, star ag® gel, wistar male rats weighing 200-300 grams.

### Extraction of Moringa Root Extract

Moringa root samples were taken from Naibonat Village, East Kupang District, Kupang Regency, East Nusa Tenggara.

Moringa root simplisia powder is made by drying moringa roots that have been cleaned and cut into small pieces. Moringa roots were dried, then pulverized and extracted using maceration method. A total of 500 grams of Moringa root simplisia powder was macerated with 96% ethanol as much as 5000 ml (1:10 ratio). Maceration was carried out for 3x24 hours while occasionally stirring. After filtering, the filtrate obtained was evaporated and a thick extract was obtained.

### Gel Formulation of Moringa Root Extract

Preparation of moringa root extract gel by developing carbopol 940 with 10 ml of distilled water at 70°C in a mortar. Add TEA, propylene glycol, glycerin, and methyl paraben which had previously been dissolved with distilled water at 75°C and crushed until homogeneous. Add Moringa root extract to the gel base and grind until homogeneous. Then add the remaining aquadest and crushed until evenly dispersed until a gel is formed. After that, the gel is stored in a closed container (**Table 1**).

**Table 1:** Moringa root extract gel formulation.

Material	Usability	Gel Formula (g)			
		I	II	III	IV
Moringa root extract	Active substance	-	5	10	15
Carbopol 940	Gelling agent	2	2	2	2
Triethanolamine (TEA)	Neutralizing agent	2	2	2	2
Propylene glycol	Cosolvent	15	15	15	15
Glycerin	Humectant	8	8	8	8
Methyl paraben	Preservative	0,2	0,2	0,2	0,2
Distilled water	Solvent	Add 100			

Note :

Formula I : Gel formula without extract concentration

Formula II : Gel formula containing 5% extract

Formula III : Gel formula containing 10% extract

Formula IV : Gel formula containing 15% extract

### Diabetic Induction and Wound Excision

The protocol and treatment of experimental animal have received Ethical Clearance approval from the Committee of Ethical Clearance for Pre-clinical Research of The Integrated Laboratory of Research and Testing, Gadjah Mada University, Yogyakarta with number 00062/X/UNI/LPPT/EC/2024.

In the diabetic wound test, rats that will be induced by streptozotocin (STZ) are fasted for 12 hours. Rats were induced with STZ at a dose of 40 mg/kgBB dissolved in 0.1 M sodium citrate buffer pH 4.0. After STZ induction, rats were checked for sugar levels using the GOD-PAP method on day 0 and day 3. Wound biopsies were performed on rats that had blood sugar levels  $\geq 200$  mg/dL.

Ketamine 80 mg/kg BW was injected intramuscularly as anesthesia. The fur on the rat's back was shaved and cleaned with 70% alcohol. Given a biopsy wound using a 4 mm diameter biopsy punch and incised using a scalpel with a wound length of 2 cm and a wound depth of 2 mm.

### Wound Healing Parameters

The wounded rats were given treatment from each control group, consisting of positive control (bioplacenton® gel for incision wounds and star ag® gel for diabetic wounds), normal control (no treatment), negative control (gel base), 5%, 10% and 15% moringa root extract gel treatment control. The application is done twice a day with an interval of 12 hours for 21 days. Wound surface area was measured at 2-day intervals on days 1, 3, 5, 7, 9, 11, 13, 15,

17, 19, and 21. Measurements of the incision wounds were taken using a digitas capiler and the results recorded. Measurements of diabetic wounds were taken using millimeter paper blocks and photographed, then analyzed using the MacBiophotonic ImageJ computer program to obtain consistent results. Determination of the percentage of wound closure is calculated using the formula.

$$P\% = \frac{do - dx}{do} \times 100\%$$

Note:

P% : Healing percentage

do : Initial wound area

dx : Final wound area

### Statistical Analysis

Data were statistically analyzed by One Way ANOVA (Analysis of Variant) test using SPSS 29.0.1.0 software program. And continued with the Student-Newman-Keuls (SNK) test to see whether there is a significant difference between each control group and moringa root extract gel treatment group.

## RESULTS AND DISCUSSION

In this study, streptozotocin (STZ) was used as an inductor of diabetes because it can damage pancreatic  $\beta$ -cells resulting in hyperglycemia.<sup>21</sup> Rats that have been induced by STZ have diabetes because they have blood sugar levels  $> 200$  mg/dL. The wound healing process is a physiological process in which living tissue cells regenerate back to their original structure before the injury.<sup>22</sup>

Based on several studies conducted previously, aqueous extracts and ethyl acetate extracts showed faster wound closure healing compared to ethanol extracts. The results of the wound healing comparison obtained are aqueous extract (90.17±0.54%) while ethanol extract (88.17%±0.47%) and ethyl acetate extract (99.87±0.42%) while ethanol extract (99.69%±0.45%).<sup>23</sup>













The effectiveness test of moringa root extract gel was carried out by applying the gel on the backs of rats that had been given wounds. The wound healing parameters observed were measuring and calculating the percentage of wound closure, observing the morphological appearance of the wound and observing the wound healing effectiveness of moringa root extract gel. The treatment of each

control group was done to compare the healing of each control group **Table 2** and **Table 3**

Wound measurements were performed using MacBiophotonic ImageJ. These measurements were taken every 2 days, starting from day 1 to day 21. The results of the percentage of healing of incision and diabetic wounds can be seen in **Table 4** and **Table 5**.



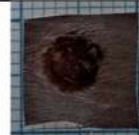

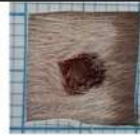
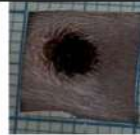





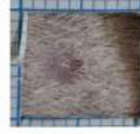
**Table 4** shows that the application of moringa root extract gel with concentrations of 5%, 10%, and 15% can heal the incision wound, respectively, wound closure reached 100% healing percentage on days 15, 13, and 11. The bioplacenton® gel group showed rapid wound closure. Whereas the untreated group and the gel base group showed prolonged wound closure.

**Table 2:** Observation of incision wound healing in each group.

Day	Group					
	Bioplacenton® gel	No treatment	Base gel	GEAK 5%	GEAK 10%	GEAK 15%
1						
21						

Note:  
GEAK : Moringa root extract gel

**Table 3:** Observation of diabetic wound healing in each group.

Day	Grup					
	Star ag® gel	No treatment	Base gel	GEAK 5%	GEAK 10%	GEAK 15%
1						
21						

Note:  
GEAK : Moringa root extract gel

**Table 4:** Percentage of incision wound healing.

Day	Wound Healing Percentage (%)					
	Bioplacenton® gel	No treatment	Base gel	GEAK 5%	GEAK 10%	GEAK 15%
1	11,17 ± 0,07	0,00 ± 0,00	4,00 ± 0,02	4,00 ± 0,02	4,00 ± 0,03	9,50 ± 0,01
3	30,00 ± 0,01	4,00 ± 0,02	11,50 ± 0,02	14,00 ± 0,02	14,00 ± 0,03	19,00 ± 0,01
5	40,17 ± 0,01	11,50 ± 0,02	22,00 ± 0,02	23,67 ± 0,02	34,50 ± 0,01	39,50 ± 0,01
7	64,83 ± 0,02	22,00 ± 0,02	31,50 ± 0,02	34,17 ± 0,02	54,33 ± 0,02	64,33 ± 0,02
9	85,17 ± 0,01	31,50 ± 0,02	42,00 ± 0,02	63,50 ± 0,02	73,33 ± 0,02	84,17 ± 0,02
11	100,00 ± 0,00	42,00 ± 0,02	51,50 ± 0,02	74,17 ± 0,02	94,50 ± 0,01	100,00 ± 0,00
13	100,00 ± 0,00	51,50 ± 0,02	71,50 ± 0,02	83,33 ± 0,01	100,00 ± 0,00	100,00 ± 0,00
15	100,00 ± 0,00	62,00 ± 0,02	82,00 ± 0,02	100,00 ± 0,00	100,00 ± 0,00	100,00 ± 0,00
17	100,00 ± 0,00	71,50 ± 0,02	91,50 ± 0,02	100,00 ± 0,00	100,00 ± 0,00	100,00 ± 0,00
19	100,00 ± 0,00	82,00 ± 0,02	100,00 ± 0,00	100,00 ± 0,00	100,00 ± 0,00	100,00 ± 0,00
21	100,00 ± 0,00	91,50 ± 0,02	100,00 ± 0,00	100,00 ± 0,00	100,00 ± 0,00	100,00 ± 0,00

Note:

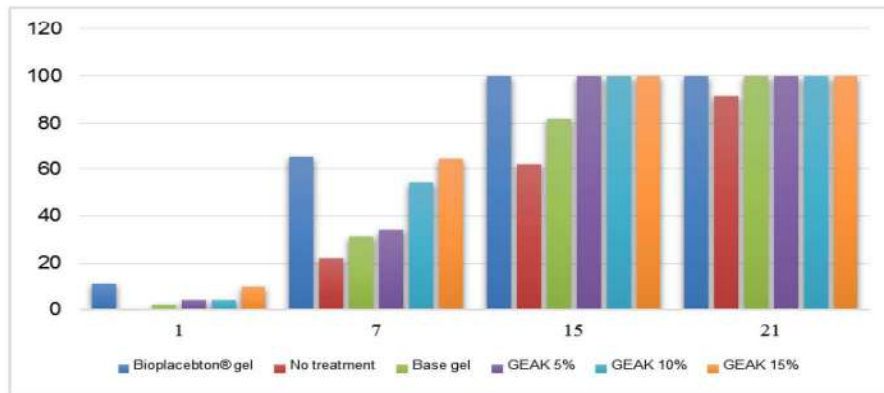
GEAK : Moringa root extract gel

**Table 5:** Percentage of diabetic wound healing.

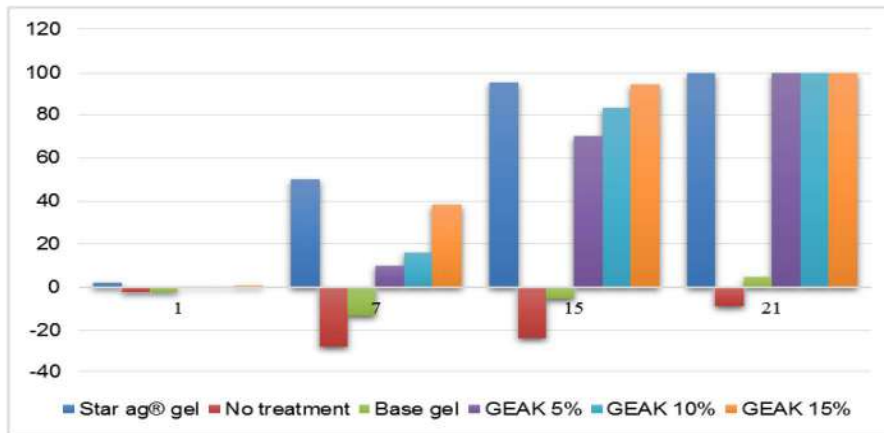
Day	Wound Healing Percentage (%)					
	Star ag® gel	No treatment	Base gel	GEAK 5%	GEAK 10%	GEAK 15%
1	1,83 ± 2,52	-2,50 ± 10,00	-2,08 ± 7,64	0,25 ± 1,73	0,50 ± 2,65	0,92 ± 0,58
3	11,25 ± 5,00	-30,00 ± 20,00	-18,75 ± 5,00	1,50 ± 3,61	1,83 ± 2,52	7,92 ± 2,89
5	25,00 ± 5,00	-28,33 ± 55,08	-15,83 ± 5,77	5,83 ± 5,77	5,83 ± 5,77	22,08 ± 7,64
7	50,00 ± 5,00	-27,50 ± 65,57	-13,33 ± 5,77	10,00 ± 10,00	15,83 ± 15,28	38,33 ± 15,28
9	65,00 ± 17,32	-26,67 ± 51,32	-12,08 ± 5,77	21,67 ± 15,28	30,83 ± 25,17	54,17 ± 15,28
11	77,50 ± 10,00	-25,42 ± 48,05	-10,83 ± 5,77	35,42 ± 23,63	48,33 ± 75,06	69,17 ± 32,15
13	90,83 ± 37,53	-24,58 ± 52,04	-8,33 ± 5,77	50,00 ± 10,00	67,50 ± 80,00	87,50 ± 36,06
15	95,83 ± 28,87	-23,33 ± 52,04	-5,42 ± 12,58	70,00 ± 26,46	83,33 ± 76,38	95,00 ± 26,46
17	100,00 ± 0,00	-12,50 ± 26,46	-2,50 ± 10,00	91,67 ± 28,87	97,50 ± 17,32	100,00 ± 0,00
19	100,00 ± 0,00	-10,83 ± 24,66	2,50 ± 10,00	98,33 ± 11,55	100,00 ± 0,00	100,00 ± 0,00
21	100,00 ± 0,00	-8,75 ± 30,41	4,58 ± 12,58	100,00 ± 0,00	100,00 ± 0,00	100,00 ± 0,00

Note:

GEAK : Moringa root extract gel.



**Fig.1:** Graph of incision wound healing percentage.



**Fig.2:** Graph of diabetic wound healing percentage.

**Table 5** shows that the application of moringa root extract gel with concentrations of 5%, 10%, and 15% can heal wounds with diabetic conditions, respectively, wound closure reached 100% healing percentage on days 21, 19, and 17. The star ag® gel group showed rapid wound closure. Whereas the untreated group and the gel base group showed prolonged wound closure. Each control group experienced different healing speeds. The greater the percent wound healing, the smaller the wound area.<sup>24</sup> This result was comparable to the previous study evaluating wound closure after treatment using *M. oleifera* hydrogel. The wound closure was significantly higher (89.76%) compared to control (45.75%) and market sample (73.38%) after day 8 of treatment<sup>25</sup>. Other study from Ali (2021) also reported that the tensile breaking strength for both 5% hexane hydrogel and 10% hexane hydrogel (152 g and 156 g, respectively) were

significantly higher compared to control placebo carbopol hydrogel and standard 5% povidone ( $p < 0.01$ )<sup>26</sup>.

The healing process of diabetic wounds takes a long time and is slightly different from that of an incision wound. This is because there are disturbances in the inflammatory phase and proliferation phase.<sup>27</sup> Where there are several physiological factors that affect the diabetic wound healing process, namely impaired blood flow and oxygenation resulting from increased blood glucose levels, decreased insulin levels, macrophage function and growth hormone, and decreased synthesis of collagen and fibronectin.<sup>14</sup>

In the wound healing stage, the inflammatory phase begins with the skin's response when it is first wounded by activating platelets which then form fibrin, causing blood to clot and stop the bleeding. This phase begins after wound administration and lasts up to 48-

72 hours.<sup>28</sup> The proliferation phase starts from the formation of new blood vessels or angiogenesis. In this phase granulation tissue, keratinocyte migration, extracellular matrix (ECM) protein formation, and myofibroblasts will cause contraction resulting in wound closure.<sup>29</sup> In diabetic wounds, the proliferation phase occurs from day 4 to day 21<sup>30</sup>. The remodeling phase begins at the transition between the late stage of the proliferation phase and the early stage of the remodeling phase, lasting up to 1 year.<sup>31</sup>

The effectiveness of moringa root extract gel for healing incision and wounds with diabetic conditions shows that the greater the concentration of moringa root extract, the faster the wound healing. This is because the higher the amount of active substance, the higher the ability to heal wounds. The compounds contained are pterygospermin, moringinine alkaloids, alkaloids, flavonoids, terpenoids, tannins and saponins which have different mechanism effects on wound healing.<sup>20</sup>

The chemical content of pterygospermin with the active substance glucosinolate benzyl isothiocyanate has activity as an antimicrobial that can prevent infection so as to increase the average wound closure.<sup>9</sup> The chemical content of moringinine alkaloids has anti-inflammatory activity that can cause constriction of blood vessels, so as to minimize the onset of inflammation, especially erythema.<sup>10</sup>

The alkaloid content plays a role in the process of strengthening collagen fibrils that are formed by preventing cell damage through DNA synthesis so that the growth of new tissue in the wound becomes faster, denser, and stronger.<sup>29</sup> The flavonoid content as an antibacterial by modifying proteins that will cause the cessation of bacterial cell metabolic activity resulting in the death of bacterial cells and can accelerate wound healing.<sup>32</sup> The content of triterpenoids as antioxidants by stopping free radicals that will cause cell damage and reduce inflammation so as to accelerate tissue repair in wounds by increasing the percentage of collagen in fibronectin cells so that the wound healing process is faster.<sup>33</sup> Tannin content as anti-inflammatory and antioxidant by inhibiting oxidant production by neutrophils, monocytes, and macrophages and can precipitate thrombin which will convert fibrinogen into fibrin so that it can stop bleeding.<sup>32</sup> The saponin content acts as an antimicrobial by preventing the growth of

microorganisms and treating skin tissue so that the wound does not experience severe infection and forms collagen through protein phosphorylation and inhibits inflammatory reactions during the initial phase.<sup>34</sup>

Data on the percentage of wound healing of incisions and diabetic wounds from day 1 to day 21 were statistically analyzed with One Way ANOVA test using SPSS 29.0.1.0 software program. The One Way ANOVA test is used to test for percentage differences between more than two groups. The data obtained is said to be normally distributed if the significant value of  $p > 0.05$  and homogeneous if the significant value of  $p > 0.05$ .<sup>35</sup>

The normality test was conducted using the Shapiro-Wilk test which showed that the data was normally distributed. And the results of the homogeneity test show that all groups of data are homogeneous. Normality and homogeneity tests have been fulfilled, so the One Way Anova test is then carried out. The results of the One Way ANOVA test showed that the data on the percentage of healing of incision and diabetic wounds were different with a significant value of  $p < 0.05$  in each wound. Furthermore, the Student-Newman-Keuls (SNK) test was conducted to see significant differences between each treatment group.

The results of the SNK test on incision wound healing showed that the administration of 15% gel had a significant difference when compared to the negative control (gel base) and no treatment. This means that Moringa root extract gel 15% has activity against wound incision healing. The 15% gel showed no significant difference with the positive control (bioplacenton® gel). This shows that the 15% gel has almost the same wound healing effectiveness as the bioplacenton® gel. The 15% gel has better wound healing effectiveness compared to the 10% and 5% gels as shown by faster healing of the incision wound.

The results of the SNK test on diabetic wound healing showed that the administration of 15% gel had a significant difference when compared to the negative control (gel base) and no treatment. This means that 15% Moringa root extract gel has activity against diabetic wound healing. The 15% gel showed no significant difference with the positive control (star ag® gel). This shows that the 15% gel has almost the same diabetic wound healing effectiveness as the star ag® gel. The 15% gel

has better wound healing effectiveness compared to the 10% and 5% gels as shown by faster diabetic wound healing.

### Conclusion

Moringa root extract gel (*Moringa oleifera*) is effective in healing incision and diabetic wounds. At 15% concentration, moringa root extract gel showed better and faster wound closure.

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## نشرة العلوم الصيدلانية جامعة أسيوط



### دراسة فعالية هلام مستخلص جذور المورينجا (مورينجا أوليفيرا) في التئام الجروح الشقوقية و السكرية

لوسيا كلاريتا ليكي سيرن<sup>١</sup> - سري هارتاتي يولياني<sup>١</sup> - فلورنتينوس ديكا أوكتا ريسوانتو<sup>٢</sup> -  
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<sup>٢</sup> قسم التحليل الصيدلاني والكيمياء الطبية، كلية الصيدلة، جامعة ساناتا دارما، يوجياكارتا ٥٥٢٨٢، إندونيسيا

<sup>٣</sup> قسم الصيدلة، كلية الصحة، جامعة سيترا بانغسا، كوبانغ ٨٥١١١، إندونيسيا

الجلد هو العضو الخارجي للجسم، حيث يغطي ويحمي السطح الكامل للجسم. تنجم الجروح الشقوقية عن قطع جسم حاد، بينما تحدث الجروح السكرية نتيجة مضاعفات مرض السكري. تستخدم هذه الدراسة مستخلص جذور نبات المورينجا (مورينجا أوليفيرا) كمادة فعالة، نظراً لعمله كمضاد للميكروبات التي تسرع من التئام الجروح، وكمضاد للالتهابات حيث يمكن ان يقلل من حدوث الالتهاب. تم صياغة مستخلص جذور المورينجا على شكل هلام، لأنه أكثر فعالية في التئام الجروح وأسهل في الاطلاق من الشكل الصيدلاني. تهدف هذه الدراسة إلى تحديد فعالية هلام مستخلص جذور المورينجا في التئام الجروح الشقوقية والجروح السكرية. تم إجراء اختبار فعالية هلام مستخلص جذور المورينجا على ست مجموعات علاجية، وهي: K1: التحكم الإيجابي (جل Bioplacenton® و جل (Star Ag®) ، K2 التحكم الطبيعي (بدون علاج) ، K3: التحكم السلبي (الهام القاعدة) ، K4: علاج بهلام تركيز ٥% ، K5: علاج بهلام تركيز ١٠% ، K6: علاج بهلام تركيز ١٥%. تم تحليل البيانات باستخدام برنامج SPSS و اختبار تحليل التباين الأحادي (One Way ANOVA). أظهرت نتائج اختبار الفعالية أن هلام مستخلص جذور المورينجا بتركيزات ٥% و ١٠% و ١٥% كان له تأثير في التئام الجروح الشقوقية و السكرية. كما أظهرت نتائج اختبار تحليل التباين الأحادي وجود اختلاف في نسبة التئام الجروح الشقوقية و السكرية بين المجموعات، مع قيمة معنوية  $p < 0.05$  لكل نوع من الجروح.