EFFECT OF MORINGA LEAF EXTRACT (Moringa oleifera L.) ON INCREDIBLE WOUND HEALING IN WISTAR STRAIN MALE RATS

Hasty Martha Wijaya[1*], Sifa Latifah Hakim[2], Rifda Naufa Lina[3], Dian Arsanti Palupi[4], Heni Setyoningsih[5]

Health Technology Institute Cendekia Utama Kudus, Indonesia

*Corresponding Author: hastymartha18@gmail.com:

Abstract. Wounds are one of the main causes that can hinder human activities in daily activities. People generally use traditional plants as medicine. Plants that affect wound healing are plants moringa (Moringa oleifera L.). The content of flavonoids, saponins, and tannins in Moringa leaves is thought to have antibacterial, astringent, and antimicrobial effects. This study aims to determine the effect of moringa leaf extract (Moringa oleifera L.) on wound healing in male Wistar rats. This study was an experimental study using RAL (completely randomized design) using 25 rats who were divided into five groups. Mice were anesthetized with 0.1 mL ketamine and then slashed using a bistro knife. In the negative control group, the rats were not given any treatment, the positive control was given 10% povidone-iodine (Betadine®), and in the treatment groups P1, P2, and P3 were given Moringa leaf extract (*Moringa oleifera*L.) with each concentration of 10%, 20% & 40%. Povidone iodine (Betadine®) and various extract concentrations were applied to much as 0.5 ml. The treatment was carried out for 10 days by measuring the length of the wound on D2, D5, and H10. The research results were analyzed using the Shapiro-Wilk normality test and homogeneity test, the results obtained were p> 0.05, which means that they were normally distributed and homogeneous. Followed by the One Way ANOVA analysis test and the results obtained were p <0.05. Then the HSD Post Hoc test was carried out, based on this test it showed that the positive control did not differ significantly from P3, which means that the 40% concentration group had the same ability to heal wounds. Therefore, the optimal concentration of Moringa leaf extract is P3 (40% extract concentration). The optimal dose is at a concentration of 40% of Moringa leaf extract (*Moringa oleifera*L.)capable of healing cuts in male white rats of the Wistar strain.

Keywords: [Moringa leaf extract, healing cuts]

BACKGROUND

In daily work, humans are always faced with hazards such as infections, toxic reagents, and electrical equipment, glasses that are used daily have the potential to experience injury risks.(Qomariah, Lisdiana & Christijanti, 2014). Wounds are one of the main causes that can hinder human activities in daily activities(Pazry et al., 2017).

In the wound healing process, a series of interactions occur between various types of cytokine-mediated cells and the extracellular matrix which are summarized in three continuous phases. The first is the inflammatory phase which is the initial phase of the wound healing process which starts from the time the wound occurs until the fifth day. The second is the proliferative phase, which occurs on day three to day fourteen where fibroblasts proliferate and synthesize collagen. The last phase is the maturation phase in the wound healing process in the form of collagen remodeling, wound contraction, and scar maturation. This phase lasts three weeks to 2 years, in this phase the formation of new collagen changes the shape of the wound(Perdanakusuma, 2007).

Povidone iodine is a local antibacterial that is effective in topical wound care by preventing inflammation caused by bacteria and spores. However, povidone-iodine is also irritating and toxic if it enters the blood vessels. Excessive use of povidone-iodine can cause skin irritation and inhibit the formation of wound granulations because the substances in povidone-iodine are also considered foreign bodies by the body because of their different composition from body cells.(Nurdiantini, Prastiwi & Nurmaningsari, 2016).

People generally use traditional plants in medicine because they are easier to obtain, cheaper, and have low side effects(Wakkary, Durry & Kairupan, 2017; Maileh Toding et al., 2016). One of the plants that are thought to affect wound healing is the leaves moringa (Moringa oleifera L.). Dwhere Moringa leaves contain flavonoids, saponins, and tannins (Leone et al., 2015).

Flavonoids are a group of the largest phenolic compounds found in nature, exhibiting biochemical activities such as antioxidants, antivirals, and antibacterials. The main flavonoids contained in Moringa leaves include m*yricetin*, *quercetin*, and kaempferol can accelerate the process of re-epithelialization of

epidermal tissue and infiltration of inflammatory cells in the wound area. Tannins are antibacterial by forming complex compounds against extracellular proteins that can disrupt the integrity of the bacterial cell membrane. Saponins have a high level of toxicity against fungi, thus helping in the wound-healing process(Pazry et al., 2017 ; Leone et al., 2015).

RESEARCH METHODS

Types of research

This type of research is experimental using a completely randomized design (CRD) and Pre-Post Control Group Design. Where in this study the grouping of mice was taken randomly after the rats were slashed. Observations and measurements of wound length were carried out before and after treatment. The treatment given is by giving Moringa leaf extract (*Moringa oleifera* L.)with various concentrations including 10%, 20%, and 40%. Furthermore, wound healing was observed on H2, H5, and H10.

Tools and materials

The tools used were rat cages, drinking bottles, razors, bistro knives, analytical scales, dropping pipettes, rulers, glass beakers, 1 cc syringes, porcelain cups, test tubes, tube racks, and volumetric flasks. The material used in this study was Moringa leaf extract from PT. Deltomed with the brand Herbana, Ethanol 70%, aqua dest, povidone-iodine 10% (Betadine ®), Ketamine, FeCl3, Mg (Magnesium), hydrochloric acid (HCL) 2N, Ferric Chloride, Nacl 0.9%, Mayer's reagent, Dragendorf's reagent, Wagner reagent, Anhydrous acetic acid, Concentrated sulfuric acid.

Phytochemical Screening

The phytochemical examination carried out was an examination of Flavonoid compounds, Saponins, Tannins, Steroids, and Alkaloids

a. Identification of Flavonoids

   Take 1 ml of Moringa leaf extract, then add Mg powder and four drops of 2% HCL. The presence of flavonoids will be indicated by a change in the color of the filtrate to red, yellow, or orange(Putra, Dharmayudha & Sudimartini, 2016).

b. Identification of Saponins

   Take 1 ml of Moringa leaf extract into a test tube then add hot water, cool, then shake for 10 seconds. After that observed changes that occur. Then 1 drop of 2N HCL was added again and the changes that occurred were observed again. A positive result when a stable foam appears for 10 minutes(Putra, Dharmayudha & Sudimartini, 2016).

c. Identification of Tannins

   Take 1 ml of Moringa leaf extract into a test tube and add 1–2 drops of 1% iron (III) chloride reagent. The presence of tannins will be indicated by a change in the color of the filtrate to green or blackish blue(Meigaria, Mudianta & Martiningsih, 2016).

d. Steroid Identification

   Take 1 ml of Moringa leaf extract into a test tube, then add 2-3 drops of anhydrous acetic acid, then stir gently for a while until dry, then add 1-2 drops of concentrated sulfuric acid and observe the color that arises. Green-blue staining indicates steroids(Meigaria, Mudianta & Martiningsih, 2016).

e. Identification of Alkaloids

   Take 1 ml of Moringa leaf extract into a test tube, then add a few drops of 2 N HCL and distilled water, then heat over a water bath for 2 minutes, then cool and filter. The filtrate used for the alkaloid test is as follows:

   a) Three drops of the filtrate were added with 2 drops of Mayer's reagent solution, then observed for the formation of a yellow precipitate.
   b) Three drops of the filtrate were added with 2 drops of Dragendorf reagent solution, then observed for the formation of an orange precipitate.
   c) Three drops of the filtrate were added with 2 drops of Wagner's reagent solution and then observed for the formation of a brownish-yellow precipitate.

The results of the phytochemical screening above were declared positive for alkaloids if a precipitate or turbidity was formed in at least two of the three experiments above.(Vongsak et al., 2013).

Grouping and Treatment of Test Animals

The test animals used in this study were 25 male white rats. Rats were acclimatized for 7 days and then shaved on their backs. Then cleaned with 70% alcohol and anesthetized with ketamine 60 mg/kgBB (Arini, 2016). Then it is sliced using a bistro knife with a length of 2.5 cm and a depth of 0.2 cm(Nayak et al., 2006). Mice were grouped into 5 treatment groups consisting of 5 rats. Each group is given multiplication as follows:

1. Group (-) : wound left
2. Group (+) : given povidone *iodine*10% (Betadine®)
3. Treatment 1 : given extractMoringa leaves a concentration of 10%
4. Treatment 2 : given extractMoringa leaves a concentration of 20%
5. Treatment 3 : given extractMoringa leaves a concentration of 40%

Giving povidone-iodine (Betadin®) is given once a day as much as 0.5 ml(Wardani, Arcinthya & Rachmania, 2017). Povidone iodine (Betadin®) was administered for 10 days(Azevedo et al., 2018). Measuring the length of the wound using a centimeter (cm) scale ruler was carried out on H2, H5, and H10.

Data analysis

The data obtained from measuring the length of the wound is then averaged. Then the percentage of wound healing is calculated using the following formula (Kumar et al., 2008):

$$\frac{(\text{Length of wound on day 0}) - (\text{Length of wound on day 0})}{\text{Length of wound on day 0)}} \text{x}100\%$$

Information:
n : measurement result ofH2, H5 and H10.

The data obtained were analyzed using the Shapiro-Wilk normality test and the Levene statistical homogeneity test. Followed by a one-way ANOVA test to see whether or not there was a significant difference between treatment groups. Then proceed with the Tukey HSD post hoc test to find out significant differences between treatment groups.

RESULTS AND DISCUSSION

The results of the phytochemical screening of Moringa leaf extract showed that it positively contained flavonoids, tannins, saponins, steroids, and alkaloids.

Table 1. Results of Phytochemical Screening of Moringa Leaf Extract

Compound	Reactor	Results	Information
Flavonoids	Mg + HCL	+	Orange Red
tannins	FeCl3 1%	+	Black Green
Saponins	HCL	+	There is foam
Steroids	Anhydrous acetic acid + concentrated sulfuric acid	+	Greenish blue
Alkaloids	Wagner	+	Yellow precipitate
	Dragendroff	+	Orange precipitate
	meyer	-	Yellow

Table 2. Observation Results of Macroscopic Wound Healing

KP	R	Wound Healing Observations			
		Time (Days)			
		H0	H2	H5	H10
(K-)	1	◦	◦•*	◦•*	•*
	2	◦	◦•*	•*	◦•*
	3	◦	◦•*	•*	•*+
	4	◦	◦•*	•*	◦•*
	5	◦	◦•*	•*	•*+
(K+)	1	◦	•*+	•*+	✓
	2	◦	◦•*	•*+	✓
	3	◦	•*+	•+	✓
	4	◦	•*+	•*+	✓
	5	◦	◦•*	•+	✓
P1	1	◦	◦•*	•*+	+
	2	◦	•*	•+	+
	3	◦	•*	•*+	+
	4	◦	•*	•*+	+
	5	◦	•*	•+	+
P2	1	◦	◦•*	•*	+
	2	◦	•*	•+	+
	3	◦	•*	•+	+
	4	◦	◦•*	•*+	+
	5	◦	◦•*	•*+	+
P3	1	◦	•	•+	+
	2	◦	•*	•+	+
	3	◦	•	•+	✓
	4	◦	•*	•+	✓
	5	◦	•	•+	+

Information :
KP: Treatment Group
R: Replication
◦ : Open Wounds
• : Erythrema (Redness)
* :Swelling
+ : The wound is starting to close
✓ : The wound closes

Based on Table 2, it is known that the results of macroscopic observations from these observations showed that H2 wound healing in all treatment groups showed almost the same results where the average wound was still open, red, and swollen. In this case, it shows that there is an inflammatory phase in which cells and proteins in the blood, endothelial cells, and fibroblasts begin to proliferate to form granulation tissue which is an early sign of healing. (Jayalandri et al., 2016).

Observation of wound healing on H5 showed that in the negative control, the wound was still open, red, and swollen. However, in the positive control, concentrations of 10%, concentrations of 20%, and concentrations of 40% showed that the wound was starting to close but redness and swelling still

occurred. Swelling of the wound is due to hyperemia and is largely due to the delivery of fluid and cells from the circulating blood to the interstitial tissues. (Luviana & Supriyanto, 2010).

Whereas in the observation of H10 wound healing the negative control showed that it still appeared reddish and swollen and some rats had visible wounds that were starting to close. This is because the negative control is not given an active substance that can accelerate wound healing. However, the wound healing process in the negative control was still ongoing with a marked decrease in the length of the wound even though it required a longer time. In the positive control, the wound was given Povidone iodine 10% to show that the wound in this group had completely closed. At extract concentrations of 10%, 20%, and 40%, the rats' wounds had begun to close. This was due to several factors that could affect wound healing. According to Arisanty (2013) wound hydration is one of the factors that influence wound healing. Moist wound conditions are very supportive in the wound healing process because in a wound that is too dry, it will produce hardened fibrin while in a wound that is too wet, it will cause damage which will worsen the wound condition. At an extract concentration of 40%, there were two rats which showed that the wound had completely closed. This is due to the ongoing remodeling phase which is marked by narrowing the size of the wound or closing the wound (Mappa, Edy & Kojong, 2013).

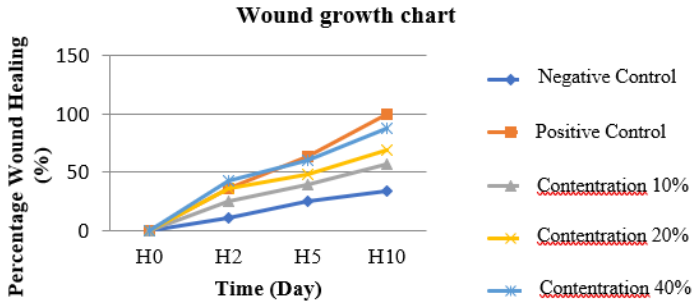

Figure 1. Wound Healing Percentage Graph

Based on table 3 the results of wound healing in the treatment group showed different results. The table shows that the results of the percentage of wound healing at H10 are in the positive control which shows the percentage value of wound healing reaching 100%, then followed by a 40% concentration of 88%, a 20% concentration of 69.6%, a 10% concentration of 57, 6% and the lowest wound healing rate was the negative control, which was 34.4%. This was because the negative treatment group was not given treatment to accelerate wound healing. However, in the negative group, the wound-healing process continued, although it was not significant. This is because the body has a natural ability, where the healing process will occur according to the body's physiological processes (Yunitasari, Alifiar & Priatna 2016).

The results of the one-way ANOVA test showed that there was a significant difference between treatment groups as indicated by a p-value <0.05. Meanwhile, the results of the HSD post hoc test showed that at H2, H5, and H10 the negative control was significantly different from the positive control, this was because the negative control was not given any treatment that could help in wound healing, so the wound healing process would take longer. compared to other treatment groups.Whereas the positive control was given 10% povidone-iodine (Betadine®) which can help in healing wounds. Povidone iodine 10%functions as a broad-spectrum antibacterial that can fight bacteria, fungi, and spores thereby accelerating the wound healing process(Khan & Naqvi, 2006).

These results also showed that the negative controls for H2, H5, and H10 were significantly different with extract concentrations of 10%, 20%, and 40%. This is because Moringa leaf extract contains compounds that can help in the wound healing process, namely flavonoid compounds. Flavonoids function as vasodilators which can improve blood flow and accelerate the formation of new blood capillaries. Anthocyanin flavonoids are antioxidant substances that have anti-inflammatory effects to accelerate the wound healing process (Prihanti, 2007).

The HSD results of the 10% and 20% concentration groups on H2 showed no significant difference from the positive control. However, on H5 and H10 it showed that the 10% and 20% concentrations were significantly different from the positive control. This situation can be caused by a decrease in anthocyanin levels as a result of a long storage process. Many factors cause anthocyanin instability

including light. Light can trigger a photochemical reaction which can open the bonds of the anthocyanin ring so that it can cause a decrease in the quality of anthocyanin. These factors can cause a decrease in the effectiveness of the number of fibroblasts and blood vessels in the treatment group(Rosa et al., 2018).

Whereas in H2, H5, and H10 it shows that the 40% concentration is not significantly different from the positive control. This is because Moringa leaf extract at P3 has the same effectiveness as the positive control in healing wounds. In addition to flavonoid compounds, saponin, and tannin compounds also play a role in wound healing. Where the role of saponin compounds as antimicrobials, reduces blood clotting and can stimulate collagen growth in the wound healing process by inhibiting excessive production of wound tissue, and has the effect of relieving pain and stimulating the formation of new cells(Igbinosa, Igbinosa & Aiyegoro 2009; Setyoadi & Sartika, 2010). Tannin compounds also function as antioxidants. which can reduce the presence of free radicals that can damage cell membranes and reduce the release of inflammatory cell mediators which means it can accelerate tissue repair in the wound healing process(Handayani & Sentat, 2016).

Table 3. Homogeneous Subsets H2

Treatment Group	1	2	3
Negative Control	11.20		
Extract Concentration 10%		25,60	
Positive Control		36,80	36,80
Extract Concentration 20%		36,80	36,80
Extract Concentration 40%			42,40

Table 4. Homogeneous Subsets H5

Treatment Group	1	2	3	4
Negative Control	24.80			
Extract Concentration 10%		39,20		
Extract Concentration 20%		48.80	48.80	
Extract Concentration 40%			60.00	60.00
Positive Control				63,20

Table 5. Homogeneous Subsets H10

Treatment Group	1	2	3
Negative Control	34,40		
Extract Concentration 10%		57,60	
Extract Concentration 20%		69,60	
Extract Concentration 40%			88.00
Positive Control			100.00

Based on observations on the homogeneous subsets table from the results of the HSD test used to determine which treatment group has the same ability as the positive control in healing wounds. In the homogeneous table, the H2 subsets show that concentrations of 10% and concentrations of 20% are in one column with the positive control, which means concentrations of 10% and concentrations of 20% have the same effectiveness as positive controls and in H2 it also shows that concentrations of 20% and concentrations of 40% are one column with a positive control, which means that the concentration of 20% and 40% has the same effectiveness in wound healing as the positive control. However, the homogeneous table for subsets H5 and H10 shows that only 40% concentration is in one column with positive control.

CONCLUSIONS

Conclusion

Based on the results of testing the effectiveness of Moringa leaf extract in healing wounds, it can be concluded that:

1. Moringa leaf extract (Moringa oleifera L.) is effective in healing wounds in Wistar rats based on macroscopic observations.
2. The optimal concentration of the ethanol extract of Moringa leaves (Moringa oleifera L.) which can heal wounds in rats is at an extract concentration of 40%.
3. Moringa leaf extract (Moringa oleifera L.) has been able to heal cuts on H10.

Suggestion

The research conducted still has many shortcomings, it is necessary to carry out further research regarding:

1. It is necessary to carry out further research and histopathological observations including the number of inflammatory cells and tissue re-epithelialization.
2. Further research is needed to make pharmaceutical preparations and toxicity tests to determine the concentration limits that are safe to use.

REFERENCES

Arisanty, IP (2013). Basic concepts of wound care management. EGC: Jakarta.

Arini, LA (2016). Testosterone replacement therapy increased the expression of androgen receptor mRNA in the prostate gland of castrated male Wistar rats (Rattus novergicus). Directory Of Open Access Journals vol 7(1), p. 83–88.

Azevedo, IM, Filho, IA, Teixeira, MMA, Moerira, M. & Medeiros, ACM (2018). Wound healing of diabetic rats treated with Moringa olifera extract. Experimental Surgery, vol. 33(9).

Handayani, F. & Sentat, T. (2016). Activity test of ethanol extract of cherry leaves (muntingia calabura L.) on the healing of burns on the skin of male white mice (mus musculus). Scientific Journal of Ibnu Sinavol 1(2).

Igbinosa, OO, Igbinosa, EO & Aiyegoro, OA (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from jatropha curcas (linn). African Journal Of Pharmacy And Pharmacology vol. 3(2).

Jayalandri, NLGL, Nangoy, E., Posangi, J. & Bara, RA (2016). Test the effectiveness of jasmine extract (jasminum sambac) on the healing of a rabbit incision wound (Oryctolagus cuniculus). Journal of E-Biomedikvol. 4(1).

Khan, MN & Naqvi, AH(2006). Antiseptics, iodine, povidone iodine, and traumatic wound cleansing. Journal Of Tissue Viability vol.16(4), p.6–10.

Kumar, MS, Kirubanandan, S., Sripriya, R. & Sehgal, PK (2008). Triphala promotes the healing of infected full-thickness dermal wounds. Journal Surgical Res. 144, 94–101.

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J. & Bertoli, S. (2015). Cultivation, genetics, ethnopharmacology, phytochemistry, and pharmacology of moringa oleifera leaves. International Journal of Molecular Sciences. p.s. 12791–12835

Luviana, LAI, An & Supriyanto. (2010). Effect of topical fracture plant sap on histopathological features and thickness of the skin keratin layer. National Seminar on Biology Education Fkip Uns

Maileh T., Fridayanti, A., Ayu, WD, Rusli, R. (2016). The effect of giving the ethyl acetate fraction of libo fruit (Ficus variegata B.) on the healing time of cuts in male white rats (Rattus norvegicus) Wistar strain. Proceedings of the National Seminar on Pharmacy vol. 4, p. 20–21.

Mappa, T., Edy, HJ, & Kojong, N. (2013). Formulation of sasaladahan leaf extract gel (peperomia pellucida (L.) HBK) and its effectiveness test against burns in rabbits (Oryctolagus cuniculus). Pharmaceutical Scientific Journal. 2(2), p. 49–56.

Meigaria, KM, Mudianta, IW, & Martiningsih. NW(2016). Phytochemical screening and antioxidant activity test of acetone extract of Moringa leaves (Moringa Oliefera). Journal of Wahana Mathematics and Science vol. 10(1), p. 1–11.

Nayak, S., Nalabothu, P., Sandiford, S., Bhogadi, V., & Adogwa, A., (2006). Evaluation of wound healing activity of allamanda cathatica. L. and Laurus nobilis. l. extracts on rats. BMC Complementary and Alternative Medicine vol. 6, p. 1–6.

Nurdiantini, I., Prastiwi, S. & Nurmaningsari, T. (2016). Differences in the effect of using 10% povidone-iodine with olive oil on lacerated wound healing. Journal Nursing News vol. 2(1), p.511–23.

Pazry, M., Busman, H., Nurcahyani, N. & Sutyarso (2017). The potential of ethanol extract of bitter gourd leaves (Momordica charantia L.) as an alternative medicine for wound healing on the backs of male mice (mus musculus L.). Agricultural research journal vol. 17(2), p. 109–16.

Perdanakusuma, DS (2007). Anatomy and physiology of the skin and wound healing. From Caring To Curing, Pause Before You Use Gauze, p. 1–8.

Putra, WDP, Dharmayudha, AAGO & Sudimartini, LM (2016). Identification of chemical compounds of ethanol extract of Moringa leaves (Moringa oleifera L.) in Bali. Indonesia Medicus Veterinus.vol. 5(5), p. 464–73.

Qomariah, S., Lisdiana & Christijanti, W. (2014). Effectiveness of broken bone extract ointment (euphorbia tirucalli) on wound healing in white rats (Rattus novergicus). Unnes Journal Of Life Science vol. 3(2): 79–86.

Rosa, SA, Adi, S., Khairani, AF & Lantika, UA (2018). Effect of yellow potato gel (solanum tuberosum L.) on the process of wound healing in mice (mus musculus). Global Medical & Health Communication vol. 6(38),p. 21–27.

Setyoadi & Sartika, DD (2010). The effect of crushed god leaves (gynura segetum) in shortening the healing time of clean wounds in white rats. Journal of Nursing Soedirmanvol. 5(3), p. 127–35.

Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y. & Gritsanapan, W. (2013). Maximizing total phenolics, total flavonoid contents, and antioxidant activity of Moringa oleifera leaf extract by the appropriate extraction method. Industrial Crops and Products vol. 44, p. 566–571.

Wakkary, JJ, Durry, M. & Kairupan, C. (2017). Effect of banana weevil sap (Musa paradisiaca var. Sapientum L. Kuntze. AAB) on the healing of cuts on the skin of Wistar rats (Rattus norvegicus). Journal of e-biomedicine (eBM) vol. 5.

Wardani, E., Arcinthya, R. & Rachmania. (2017). Activity test of ethanol extract and ethyl acetate extract of red betel leaves (piper cf. Fragile. Benth) on open wound healing in rats. Pharmaceutical Mediavol. 14(1): 43–60.

Yunitasari, D., Alifiar, I., & Priatna, M. (2016). Activity Test of Jengkol Leaf Ethanol Extract (Pithecellobium Lobatum Benth). Journal of Pharmaceutical Sais and Practical vol. 2.