EFFECTIVENESS OF MANALAGI MANGGO LEAF EXTRACT GEL (*Mangifera indica L.*) ON CUTTING WOUND HEALING IN MALE RABBITS NEW ZEALAND WHITE

Niken Luthfiyanti[1]*, Anif Muktamaroh[2], Isna Nur Khasanah[3]
Universitas Duta Bangsa Surakarta / Faculty of Health Sciences / Bachelor of Pharmacy Education Program[123]
*Correspondence Email : niken_luthfiyanti@udb.ac.id

ABSTRACT

Wounds are pathological conditions that occur in skin tissue and mucous membranes of other tissues. Mango leaves (Mangifera indica L.) contain alkaloids, flavonoids, tannins and saponins which have an effect on wound healing. The purpose of this study was to determine the effectiveness of mango leaf extract gel concentration (Mangifera indica L.) on wound healing in rabbits. The test animals were divided into five treatments, namely positive control, negative control, and gel preparation with 3 variations of extract concentration, namely formula I (10%), formula II (15%), and formula III (20%). This research is an experimental study with observations for 14 days. The statistical test uses the Anova test and the Post-Hoc test. The average healing time of the positive control and negative control groups with mango leaf extract gel (Mangifera indica L.) concentrations of 10%, 15%, and 20% had a significant difference with a sig 0.000 result (P<0.05). Manalagi mango leaf extract gel (Mangifera indica L.) provides the best wound healing effect at a concentration of 20%.

KEYWORDS Gel, Manalagi Mango Leaf Extract, Wounds

INTRODUCTION

Wound is one of the processes of damage to the anatomical structure of the skin caused by sharp object scratches, impact on blunt objects, bites animals, chemicals, electric shocks, and explosions (Sjamsuhidajat, 2005). There are various types of wounds, one of which is an incision. Cut is a form of damage or loss of body tissue caused by a sharp object. The characteristics of an incision wound are an open wound, pain, the length of the wound is greater than the depth of the wound. Wounds that occur when If left unchecked, an infection will occur so that the microorganisms that are in around the wound can enter

the body parts of the skin, connective tissue, muscles, nerves, blood vessels and bone membranes (Ningsih *et al.*, 2015).

The use of chemical drugs has side effects for its use. Side effects that often arise include itching, redness, irritation, and causing swelling in the wound area (Imamah, 2017). Therefore, to reduce the occurrence of risks and side effects from the use of drugs containing chemicals and to make it easier for people to obtain traditional medicine, Manalagi mango leaves are used (*Mangifera Indica L.*). Manalagi mango leaves contain alkaloids, saponins, tannins, and flavonoids (Risa *et al*.,2018).

Seeing that manalagi mango leaf compounds have benefits for various treatments, especially for wound healing, this study aims to determine the potential of manalagi mango leaf extract gel (*Mangifera Indica L.*) in wound healing in male rabbits.

RESEARCH METHOD

Tools and materials

The tools used in this study were handscoon, mask, blender, measuring cup, erlenmeyer, filter paper, bisturi, rabbit cage, hair trimmer, scales, gauze, dropper pipette, rotary evaporator, scissors, viscometer, clipper, moisture balance, oven .

The materials used included manalagi mango leaves, 96% ethanol, male rabbit, carbopol, propylene glycol, triethanolamine, methyl paraben, aqua distillata, bioplacenton as positive controls.

Making simplicia of manalagi mango leaves

Mango leaves are picked in the Suruh area, cleaned of dirt and washed with running water until clean then drain. Dry the mango leaves in the following way air dry without direct sun exposure. After the dry mango leaves in the blender until it becomes powder. Furthermore, the mango leaf powder was sifted using a 40 *mesh* sieve until a fine powder of mango leaves is obtained (Januarti *et al*., 2023).

Manufacture of manalagi mango leaf extract

Extracting manalagi mango leaf powder by maceration method using 96% ethanol solvent and covered using aluminum foil for 5 days with occasional stirring. The extract is evaporated in a rotary evaporator at a temperature below 60^0C so that a concentrated extract of mango leaves is obtained and then the yield is calculated from the concentrated extract (Prasongko *et al*., 2020).

Standardization of simplicia and extracts

Simplicia powder and extract samples were standardized including ethanol-free test, moisture content determination test, and drying shrinkage determination test.

Phytochemical Screening

Manalagi mango leaf extract was subjected to phytochemical screening including alkaloid test, flavonoid test, tannin test, and saponin test.

Formulasi sediaan gel ekstrak daun mangga manalagi

Table 1. Modified Formulation of Mango Leaf Extract Gel Preparations Source: (Kaban *et al.*, 2022)

Material	F0 (%)	F1 (%)	F2 (%)	F3 (%)	Usage
Mango leaf extract	-	10	15	20	Active substance
Basis					
Karbopol 940	0,5	0,5	0,5	0,5	*Gelling agent*
Trietanolamin	2	2	2	2	Emulsifying

					agent
Propilenglikol	15	15	15	15	Humectants
Metilparaben	0,2	0,2	0,2	0,2	Presertative
Aquadest ad	100	100	100	100	Solvent

Evaluation of gel preparations

Evaluation of the gel preparations included organoleptic tests, homogeneity tests, pH tests, spreadability tests, and viscosity tests.

Preparation and treatment of test animals

This research has passed the ethical test by the Moewardi Hospital Health Research Ethics Commission number: 502 / III / HREC / 2023. A total of 4 test animals were adapted (aclimatized) in a new environment (laboratory) for 7 days. After that, the rabbit's fur was shaved clean and marked on the back as a treatment marker.

The incision was made according to the mark with an incision length of 2 cm and a depth of 0.2 cm. After incisions were made to all the test animals, treatment was carried out according to the signs of the test group and the control group. Treatment is given 2 times a day in the morning and evening (Putrianirma *et al*., 2019).

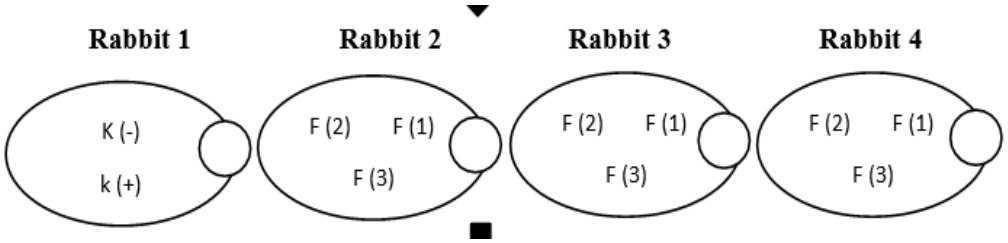

Figure 1. Treatment Plan for Rabbits

Wound healing percentage

The parameter in this study is to see a decrease length of wound, and time of disappearance of erythema. Length measurement healing for cuts, namely using caliper for 14 days (Megawati *et al*., 2020). The data to be analyzed namely The percentage of wound healing is obtained through measurement length of the incision. Measurements are taken once per day (Prasongko *et al*., 2020).

The formula for calculating the percentage of long wound healing:

$$p\% = \frac{\mathrm{d0} - dx}{\mathrm{d0}}\ x\ 100\%$$

Information :

p% : percentage of wound healing
d0 : initial wound diameter
dx : wound diameter on the day of observation

Data analysis

The research data obtained were in the form of quantitative data which were analyzed using IBM SPSS Statistics ver. software. 22 and qualitative data were analyzed descriptively.

RESULT AND DISCUSSION

Manalagi mango leaves are taken at 09.00-12.00 WIB, this is done because in the morning before noon photosynthesis occurs. Photosynthesis will produce nutrients in the form of carbohydrates and proteins that affect the biosynthesis of secondary metabolites. The biosynthetic pathway of secondary metabolites through pathways outside the biosynthesis of proteins and carbohydrates (Julianto, 2019). After picking manalagi mango leaves, dry sorting is carried out to separate the plants from the impurities. As much as 5 kg of Manalagi mango leaves obtained were then washed using running water, with the aim of removing soil and dirt and then dried for 5 days (Wahyuni & Semboh, 2021). Manalagi mango leaf simplicia drying is carried out in aerated way to reduce the water content and minimize the active compounds damaged by high temperatures (Himawan *et al.*. 2017). After drying, dry sorting is carried out to separate it from impurities that are not used. Simplisia is made into powder using a herb grinder. Furthermore, the simplicia powder was sieved using sieve no. mesh 40 (Himawan *et al.*, 2017).

As much as 500 grams of simplicia powder from mango manalagi leaves was macerated and re-macerated for 5 days with 96% ethanol solvent. The maserate obtained is evaporated using a rotary evaporator at a temperature of 50°C because the compound which is thermolabile will be damaged at temperatures above 60°C. The yield of the extract produced was 120 grams with a yield of 12%. The extract yield obtained has met the requirements according to the standard by the 2017 Indonesian Herbal Pharmacopoeia which states that the extract yield must not be less than 19%. The simplicia powder and extracts obtained were then characterized with the aim of seeing the guarantee and uniformity of simplicia quality to meet applicable standards (Herbal Pharmacopoeia, 2017). Based on Table 2, the characterization results show that the simplicia and extracts obtained in this study have met the requirements of the applicable standards.

Table 2. Standardization of simplicial powders and extracts

Standardization	Results	Requirement
Simplicia Powder Samples		
Drying shrinkage test	0,22%	<10%
Test the water level	9,38%	<10%
Extract Sample		
Ethanol free test	No ester odor	Odorless ester when heated after addition of acetic acid and sulfuric acid
Drying shrinkage test	0,19%	<10%
Test the water level	5,44%	<10%

Phytochemical Screening

Table 3. Phytochemical Screening of Manalagi Mango Leaf Extract

Sample	Phytochemical test	Reactor	Standard	Results
Manalagi Mango Leaf Extract	Alkaloid	Dragendrof	Brick precipitate	+
	Flavonoid	Concentrated HCl powder Mg	+Yellow, orange, dark red	+
	Tanin	FeCl3	Black Green	+
	Saponin	HCl 2N	Therefore	+

To see the compounds present in mango manalagi leaf extract, a phytochemical screening was carried out using the tube method. Based on Table 3, manalagi mango leaf extract contains alkaloids, flavonoids, tannins, and saponins. Manalagi mango leaves are known to contain compounds that have medicinal benefits such as: alkaloids, saponins, tannins, and flavonoids (Risa *et al.*, 2018).

Evaluation of Gel Preparations

Tabel 4. Evaluation of Manalagi Mango Leaf Extract Gel Preparations

Category	Formula		
	Gel 10%	**Gel 15%**	**Gel 20%**
Organoleptis Test			
Form	Gel	Gel	Gel
Color	Light yellow	Yellow	Orange
Smell	Mango leaves	Mango leaves	Mango leaves
Homogenity test	Homogeneous	Homogeneous	Homogeneous
pH test	5,33	5,39	5,40
Spreadability test	5,6	5,3	5,1
Viscosity test	9817,6 mPas	9818,7 mPas	9819,9 mPas

Organoleptic observations of gel preparations in table 4 showed that before and after storage did not experience significant changes, namely with a light yellow to orange color and a distinctive smell of mango leaves. This shows that in this parameter the preparation is said to be either before or after storage, or the components in the preparation during storage do not experience a reaction between one material and another, so there are no signs of a reaction from a change in color. The addition of extracts can affect the gel preparations organoleptically both in color and smell (Lubapepita *et al*.,2021).

Observation of homogeneity test results at all concentrations of the preparation showed that there were no solid particles in it or the gel was uneven and still clumped. The homogeneity test aims to see the active substance spreads perfectly in the gel preparation (Hidayah, 2019).

The results of observing the pH (table 4) of the manalagi mango leaf extract gel correspond to the pH of the gel preparation used on the skin, which is 4.5-6.5 (Dhimas, 2022). Observation of the pH of the Manalagi mango leaf extract gel preparation used universal pH.

The spreadability test of the preparation was carried out to determine the amount of force needed by the gel to spread on the skin or to determine the ability to spread the gel preparation when applied to the skin. The criterion for good spreading power is 5-7 cm (Dhimas, 2022). The test results for the spreadability of the three formulas all met the physical quality requirements or parameters for good spreadability of gel preparations.

Viscosity test was carried out to determine the thickness of the gel. A gel that is not too runny or not too thick is a good feature. Gel base viscosity was measured at 0.1 rpm for 30 minutes with viscometer brokfield. A good viscosity value for gel preparations is 500-10,000 mPas (Rahmatullah *et al*.,2020). The results of the viscosity test of the three formulas all met the requirements for a good gel preparation viscosity.

Cut Wound Healing Activity Test

Table 5. Wound Healing Activity Test Precentage 100% Viewed from Healing Time

Replication	Time (Days)				
	Gel 10 %	**Gel 15%**	**Gel 20%**	**control +**	**control -**
1	11	10	9	8	12
2	11	10	8	-	-
3	11	11	9	-	-
Rerata	11	10,3	8,6	8	12

Tabel 6. *Post-hoc* Anova test

Treatment	Gel 10%	Gel 15%	Gel 20%	Control Positive	Control Negative
Gel 10%		0.029*	0.000*	0.000*	0.026*
Gel 15%			0.040*	0.000*	0.000*
Gel 20%				0.011*	0.000*
Control Positive					0.000*
Control Negative					

*: p-value < 0.05 (there is a significant difference)

The injured blood vessel tissue will be cut off resulting in bleeding. Hemostatic reactions are the body's first reaction when bleeding occurs, namely stopping bleeding by activating coagulation factors both intrinsic and extrinsic (Primadina & Perdanakusuma, 2019).

The wound healing process is a process that involves cellular and biochemical responses both locally and systemically (Primadina & Perdanakusuma, 2019). The wound healing process includes the inflammatory, proliferative, and maturation phases. In the initial phase, there is an inflammatory phase after an injury so that there is a hemostatic reaction aimed at removing dead tissue and preventing infection by pathogenic microbes (Primadina & Perdanakusuma, 2019).

On the first day of observation, there was an inflammatory reaction marked by swelling and redness. According to Suryadi *et al*., (2013) this inflammatory phase is characterized by swelling, heat, pain, and redness. On day 3 to day 14 it has entered the proliferative phase. This phase is marked by reduced inflammation, redness, appearance of fibroblast cells, and the formation of new blood vessels. The formation of new blood vessels is also known as angiogenesis.

Based on Table 5, the gel concentration of 20% has the fastest healing time compared to gel concentrations of 10% and 15%, with an average wound healing of 8.6 days. Anova test in Table 6 aims to determine the difference between the five treatment groups. Anova test results with a significance value of 0.000 which indicates a value less than 0.05. These results indicate that there are differences between the five treatment groups.

Manalagi mango leaves contain compounds that can accelerate the healing of cuts including alkaloids, flavonoids, tannins, and saponins. Tannins can be used to enhance the wound healing process through several mechanisms, namely scavenging free radicals, encouraging wound contraction, increasing the formation of capillaries, and fibroblast proliferation. This shows that tannin compounds help the healing process in the proliferative phase (Siahaan *et al.,* 2017; Kim *et al.,* 2013). Saponin compounds can increase the expression of factors that are relevant to proliferation thereby encouraging the re-epithelialization of wounds. In addition, in the initial phase it effectively inhibits the inflammatory reaction (Kim et al., 2013; Rathee *et al.,* 2014). Flavonoids have a working mechanism as an anti-inflammatory so that they have a beneficial effect on the wound healing process, because these compounds increase fibroblast migration and proliferation and exudative inflammation (Antunes-Ricardo *et al.*, 2015). Most of the flavonoids exert antibacterial activity and help control infection (Orlowski *et al.*, 2018).

The results of this study are in accordance with previous studies where the ethanol extract of mango leaves (*Mangifera Indica L.*) has wound healing activity (Risa *et al.,*

2018). Based on the results of the research analysis showed that the preparation of mango leaf extract gel (*Mangifera Indica L.*) 20% concentration can be used as an alternative medicine for wound healing because there is a significant difference in the intervention with the negative control group.

CONCLUSION

Preparation of mango leaf extract gel (*Mangifera indica L.*) has wound healing activity in male rabbits. Based on the wound healing time, mango leaf extract gel preparation with a concentration of 20% had the fastest time to heal cuts compared to other concentration gel preparation groups.

The formulation of the mango leaf extract gel preparation meets the physical quality tests of the gel preparation, including organoleptic test, pH test, spreadability test, homogeneity test and viscosity test.

REFERENCES

Antunes-Ricardo M, Gutiérrez-Uribe J, SernaSaldívar SO. (2015) 'Anti-inflammatory glycosylated flavonoids as therapeutic agents for treatment of diabetes-impaired wounds'. *Curr Top Med Chem*, 15(23), pp. 2456-2463. doi: 10.2174/1568026615666150619141702

Dhimas T., (2022). Formulasi Dan Uji Anti Jerawat Sediaan Gel Ekstrak Daun Asam Jawa (*Tamarindus indica L*) Terhadap Bakteri Staphylococcus epidermidis ATCC 12228. Universitas Duta Bangsa Surakarta

Farmakope Herbal Indonesia. 2017. Edisi ll. Kementerian Kesehatan Republik Indonesia.

Hidayah nur. (2019). formulasi sediaan gel ekstrak air bunga rosella (hibiscus sabdariffa L) terhadap penyembuhan luka sayat pada kelinci (oryctolagus cuniculus). *Progress in Retinal and Eye Research, 561*(3), S2–S3.

Himawan, H. C., Pramono, P., & Resti, D. A. (2017) 'Uji Farmakologis Ekstrak Kental Daun Meniran (Phyllanthus niruri Linn) Untuk Membantu Penyembuhan Luka Sayat Pada Tikus Putih Jantan (Rattus norvegicusstrain Sprague-Dawley)'. *Jurnal Farmamedika (Pharmamedica Journal)*, 2(1), pp. 30–39. doi: 10.47219/ath.v2i1.30

Imamah, N. T. (2017) 'Pengaruh Hydrogel Centellaasiatica untuk Penyembuhan Luka Insisi'. *Jurnal Ilmiah Sehat Bebaya*. 1(2), pp. 125-131.

Januarti, I. B., Waluyo, K., Ningsih, W., & Sholeh, A. B. (2023*).* Uji aktivitas sediaan gel ekstrak daun sirih (*piper betle L* .) terhadap penyembuhan luka sayat pada kelinci (*oryctolagus cuniculus*) activity test of betel leaf extract gel (*piper betle*) toward on wound healing In rabbits(oryctolagus cuniculus). 8*(1), 229–240.*

Kaban, V. E., Nasri, N., Syahputra, H. D., Fitri, R., Rani, Z., & Lubis, M. F. (2022). Formulasi Sediaan Gel dari Ekstrak Metanol Biji Alpukat (*Persea americana Mill.*) Sebagai Penyembuh Luka Sayat Pada Tikus Jantan (*Rattus norvegicus*). *Herbal Medicine Journal*, *5*(2), 48–54. https://doi.org/10.58996/hmj.v5i2.50

Kim, Y. S., Cho, I. H., Jeong, M. J., Jeong, S. J., Nah, S. Y., Cho, Y. S., Kim, S. H., Go, A., Kim, S. E., Kang, S. S., Moon, C. J., Kim, J. C., Kim, S. H., & Bae, C. S. (2013) 'Therapeutic Effect of Total Ginseng Saponin on Skin Wound Healing'. *Journal of ginseng research*, 35(3), pp. 360– 367. doi: 10.5142/jgr.2011.35.3.360

Lubapepita Triananda, A., & Wijaya, A. (2021). Formulasi dan uji fisik sediaan gel ekstrak daun petai cina (Leucaena leucocephala (Lam.) De. Wit) dengan basis hydroxyl propyl methyl cellulose (HPMC). *Jurnal Kefarmasian Akfarindo*, 29–36. https://doi.org/10.37089/jofar.vi0.100

Megawati, S., Nur'aini, N., & Kurniasih, D. (2020). Uji efektivitas gel ekstrak etanol 96% daun singkong (manihot esculenta crantz.) pada penyembuhan luka sayat kelinci jantan galur new zealand white. *Jurnal Farmagazine*, *7*(1), 1. https://doi.org/10.47653/farm.v7i1.159

Ningsih, S., Paturusi, A., & K, N. R. A. (2015). Uji efek penyembuhan gel ekstrak daun jarak merah (jatropha gossypifolia linn) terhadap luka sayat pada kelinci. *Jurnal Farmasi UIN Alauddin Makassar*, *3*(3), 104–110.

Prasongko, E. T., Lailiyah, M., & Muzayyidin, W. (2020). Formulasi dan uji efektivitas gel ekstrak daun kedondong (spondias dulcis F .) terhadap luka bakar pada tikus wistar (rattus novergicus). *Jurnal Wiyata S1 Farmasi, Fakultas Farmasi ,Institut Ilmu Kesehatan Bhakti, Kesehatan Bhakti Wiyata*, *7(10*(2355–6498), 27–36.

Primadina, N., Basori, A., & Perdanakusuma, D. S. (2019)'Proses Penyembuhan Luka Ditinjau dari Aspek Mekanisme Seluler dan Molekuler'. Qanun Medika - *Medical Journal Faculty of Medicine Muhammadiyah Surabaya*, 3(1), pp. 31. doi: 10.30651/jqm.v3i1.2198

Putrianirma, R., Triakoso, N., Yunita, M. N., Yudaniayanti, I. S., Hamid, I. S., & Fikri, F. (2019) 'Efektivitas Ekstrak Daun Afrika (Vernonia amygdalina) Secara Topikal Untuk Reepitelisasi Penyembuhan Luka Insisi Pada Tikus Putih (Rattus novergicus)'. *Jurnal Medik Veteriner*, 2(1), pp. 30. doi: 10.20473/jmv.vol2.iss1.2019.30-35

Rahmatullah, S., Slamet, Ningrum, W. A., & Dewi, N. K. (2020). Formulasi Dan Evaluasi Sediaan Gel Hand Sanitizer Sebagai Antiseptik Tangan Dengan Variasi Basis Karbopol 940 Dan TEA. *Pharmaceutical Scientific Journal*, *3*(3), 189–194

Rathee P, Chaudhary H, Rathee S, Rathee D, Kumar V, Kohli K. (2014)'Mechanism of action of flavonoids as anti-inflammatory agents: a review'. *Inflamm Allergy Drug Targets*, 8(3), pp. 229-235. doi: 10.2174/18715280978868102

Risa, A. M., Pantiwati, Y., Mahmudati, N., Husamah, H., & Miharja, F. J. (2018). Daun mangga (Mangifera indica L): potensi baru penyembuh luka sayat. *Biota*, *11*(2), 96–106. https://doi.org/10.20414/jb.v11i2.128

Siahaan, M. S. Y., Pangkahila, W., & Aman, I. G. M. (2017) 'Gel ekstrak daun meniran (*Phyllanthus niruri*) meningkatkan epitelisasi penyembuhan luka pada kulit tikus putih jantan galur Wistar (*Rattus norvegicus*)'. *Jurnal Biomedik (JBM)*, 9(1). doi: 10.35790/jbm.9.1.2017.15314

Sjamsuhidajat R, Jong WD (2005). *Buku Ajar Ilmu Bedah.* EGC. Jakarta. 2005

Suryadi, I. A., Asmarajaya, A. G., & Maliawan, S. (2013) *Proses Penyembuhan dan Penanganan Luka*. Denpasar: Bagian/SMF Ilmu Penyakit Bedah Fakultas Kedokteran Universitas Udayana/Rumah Sakit Umum Pusat Sanglah Denpasar

Wahyuni, Aliah, A. I., & Semboh, E. (2021) 'Formula Gel dan Uji Efektivitas Ekstrak Etanol Daun Meniran (Phyllanthus niruri L.) terhadap Penyembuhan Luka Sayat pada Kelinci Jantan (Oryctolagus cuniculus)'. *Media Kesehatan Politeknik Kesehatan Makassar*, XVI(1), pp. 1–15. https://doi: 10.32382/medkes.v16i1.1798