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THE EFFECTIVENESS ASSAY OF HEALING INCUTIONS OF NANOSTRUCTURED LIPID CARRIERS (NLCS) ETHANOL EXTRACT OF *MORINGA* LEAF (*Moringa oleifera*) ON WHITE RATS (*Rattus norvegicus*)

UJI EFEKTIVITAS PENYEMBUHAN LUKA SAYAT SEDIAAN NANOSTRUCTURED LIPID CARRIERS (NLCS) EKSTRAK ETANOL DAUN KELOR (*Moringa oleifera*) TERHADAP TIKUS PUTIH (*Rattus norvegicus*)

Bambang Wijianto*, Liza Pratiwi
Pharmacy, Faculty of Medicine, Universitas Tanjungpura, Prof. Dr. Hadari Nawawi, Pontianak
**Corresponding author:* bam.wijianto@pharm.untan.ac.id

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Abstract

Wounds are disruptions of tissue integrity that impair normal physiological function. Nanostructured Lipid Carriers (NLCs) are lipid-based nanosystems that enhance the delivery of bioactive compounds. *Moringa oleifera* leaves contain bioactive constituents, including isothiocyanates and cytokinins (zeatin), which exhibit antibacterial activity and promote cell proliferation, potentially accelerating wound healing. This study aimed to evaluate the wound-healing effectiveness of NLCs containing *Moringa oleifera* leaf ethanol extract. An acute open-incision wound model was established in rats, divided into five groups: normal control, betadine ointment (positive control), NLC base (negative control), extract gel, and NLCs containing 5% *Moringa oleifera* leaf ethanol extract. Treatments were applied topically, and wound healing was monitored by measuring wound area reduction with ImageJ. The percentage of wound closure and area under the curve (AUC) values were calculated and analyzed using one-way ANOVA. Both the extract gel and NLC formulations accelerated wound healing compared with the positive and negative controls. Complete wound closure was achieved on day 8 in the treatment groups, whereas the positive and negative controls reached complete closure on days 10 and 14, respectively. These findings indicate that NLCs containing *Moringa oleifera* leaf ethanol extract have potential as an effective topical formulation for promoting wound healing.

Keywords: *Moringa oleifera*; NLCs; Topical; Wound healing

Abstrak

Luka merupakan kerusakan integritas jaringan yang mengganggu fungsi fisiologis normal. Nanostructured Lipid Carriers (NLCs) adalah sistem penghantaran berbasis lipid berukuran nano yang dapat meningkatkan efektivitas senyawa bioaktif. Daun kelor (Moringa oleifera) mengandung senyawa bioaktif, seperti isothiocyanate dan sitokinin (zeatin), yang memiliki aktivitas antibakteri dan mampu meningkatkan proliferasi sel sehingga berpotensi mempercepat penyembuhan luka. Penelitian ini bertujuan mengevaluasi efektivitas penyembuhan luka dari sediaan NLC yang mengandung ekstrak etanol daun kelor. Model luka sayat akut dibuat pada tikus yang dibagi menjadi lima kelompok, yaitu kontrol normal, salep Betadine (kontrol positif), basis NLCs (kontrol negatif), gel ekstrak, dan NLCs yang mengandung 5% ekstrak etanol daun kelor. Sediaan diaplikasikan secara topikal, kemudian proses penyembuhan luka diamati melalui pengukuran luas area luka menggunakan ImageJ. Persentase penutupan luka dan nilai area under the curve (AUC) dihitung dan dianalisis menggunakan uji One-Way ANOVA. Hasil penelitian menunjukkan bahwa gel ekstrak dan formulasi NLCs mempercepat penyembuhan luka dibandingkan dengan kontrol positif dan kontrol negatif. Penutupan luka mencapai 100% pada hari ke-8 pada kelompok perlakuan, sedangkan kontrol positif dan kontrol negatif masing-masing mencapai penutupan luka sempurna pada hari ke-10 dan ke-14. Temuan ini menunjukkan bahwa NLCs ekstrak etanol daun kelor berpotensi sebagai formulasi topikal yang efektif untuk mempercepat penyembuhan luka.

***Kata Kunci**: Moringa oleifera; NLCs; Penyembuhan luka; Topikal*

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INTRODUCTION

Skin is the body's outermost layer, which protects the inner body from various disorders. The skin sometimes experiences disturbances such as friction or punctures, which can damage it (Kaban et al., 2022). Wounds are the process of damaging part of the skin tissue and the anatomical function of the skin (Ginting et al., 2022; Tamuntuan et al., 2021). Wounds are often experienced by everyone, from minor to serious injuries. Wounds can occur due to several factors, such as sliding on a rough surface, blunt or sharp object trauma, chemical exposure, or animal bites (Watung et al., 2020). Cuts are injuries resulting from incisions or abrasions caused by a sharp object. A cut wound is characterised by being open, eliciting a pain response, and having a length that exceeds its depth. The principle in treating cut wounds is to clean the wound area to prevent infection because open skin makes it easy for microorganisms to grow, then stop the bleeding by closing the wound and giving the remaining epithelium a chance to proliferate and cover the wound surface (Agust & Asfi, 2018)

The wound-healing process entails the restoration of compromised tissue. Wound healing is the process of restoring anatomical continuity and function (Megawati et al., 2020). Wound healing involves various intricate processes due to the continuous occurrence of numerous bio-cellular and biochemical activities. The body will try to restore damaged tissue components by forming new and functional structures like the previous state (Liana & Utama, 2018). Wound healing takes place in 3 primary phases, namely, the inflammatory phase, the proliferation phase, and the maturation or remodeling phase (Aponno et al., 2014). Traditional medicine is increasingly popular in this modern era; with advances in science, many people are turning to traditional medicine. *Moringa* leaves are a natural component that facilitates wound healing.

Moringa leaves contain flavonoid compounds, especially quercetin, their most significant component (Vergara-Jiménez et al., 2017), followed by kaemferol and rutin (Mthiyane et al., 2022). Research shows that 100 g of wet simplicia *Moringa* leaves contains 384.61 mg of quercetin (Vergara-Jiménez et al., 2017). The 96% ethanol extract form shows that the flavonoid content of *Moringa* leaves is 13.15 ± 0.47%, while the 70% ethanol extract is 5.53%. The active secondary metabolite compounds of *Moringa* leaves include saponins, tannins, flavonoids, and alkaloids, which can accelerate wound closure. Wounds and acts as an anti-inflammatory with the presence of pterygospermin and moringinine alkaloid content where this substance will help vasoconstriction in blood vessels thereby minimizing signs of inflammation. Apart from that, *Moringa* leaves can also act as an antibiotic because they contain pterygospermin, which contains the active substance glucosinolate, 4-alpha-L-rhamnosyl oxybenzyl isothiocyanate (Pareek et al., 2023).

Alkaloids play a role in the wound healing process, namely in the initial phase, by stimulating the formation of fibroblast precursors. Increased fibroblast formation will increase collagen production in wound tissue (Broughton et al., 2006). Alkaloids also act as antibacterials by disrupting the peptidoglycan components in bacterial cells so that the cell layer ultimately does not form and causes cell death (Yan et al., 2021). Saponins play a role in wound healing by stimulating fibronectin production by fibroblasts and changing gene expression of the TGF-β receptor. Fibronectin stimulated by fibroblasts causes many fibroblasts to migrate into the wound gap, causing more collagen to be synthesized by fibroblasts. TGF-β, together with platelet derived growth factor (PDGF), converts fibrinogen into fibrin for the wound healing process (Kanzaki et al., 1998; Patel et al., 2019). Saponin also acts as an antiseptic by killing germs or preventing the growth of microorganisms in wounds so that the wounds do not experience serious infections (Cankaya, 2021).

Proper wound treatment involves topical applications that adhere to the skin's surface (Megawati et al., 2020; Tamuntuan et al., 2021). Topical formulations exhibit limitations, expressly limited bioavailability, and inadequate drug penetration properties. Nanostructured Lipid Carriers (NLCs) constitute a component of the nanotechnology framework (Elmowafy & Al-Sanea, 2021). The NLCs system enhances the capacity of active substances to permeate the stratum corneum and reach the epidermis. It can effectively enhance the bioavailability of active compounds in the skin and target skin. The NLCs system has additional benefits: it enhances the physicochemical stability of active substances, hydrates the skin in vivo due to its excellent occlusiveness, and imparts an emollient effect on the skin owing to its lipid content. NLCs consist of solid and liquid lipids, with a

predominance of solid lipids (Arabestani et al., 2024; Garcês et al., 2018). No study has been conducted on the formulation of NLCs utilizing *Moringa* leaf extract for the treatment of cut wounds; therefore, the author intends to investigate the efficacy of these preparations in promoting wound healing—NLCs of ethanol extract from *Moringa* leaves.

MATERIALS AND METHODS

The tools for this research are glassware (pyrex®), sieve (mesh number 18), stirring rod (pyrex®), bunsen, evaporator (Buchi), grinder (ACE-Klaz Cg9100), porcelain cup, shaving (Gillette), Buchner funnel (Schott), watch glass (pyrex®), test animal cage, filter paper, mortar, analytical balance (Ohaus), oven (Modena), water bath (Memmert UN 55), dropper pipette (pyrex®), ruler, iron plate 2 cm diameter, ointment pot, rotary evaporator (Buchi R-100 9230 Flawil), scapel number 11, 1cc syringe, and stamper. The material used is *Moringa* leaves in fresh condition, collected from Merdeka Street, Pontianak, West Kalimantan. Samples determined by No.023/A/LB/F.MIPA/UNTAN/2021 at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Tanjungpura. For the white Rat test animals, this research was conducted ethically by No.10707/UN22.9/PG/2024. Besides that, alcohol pads, aquadest (Dwicentra), ethanol 96% (Merck), ether (Merck), betadine ointment, sterile gauze, glyceryl monostearate (Evonik), flaxseed oil, tween 20, propylenglikol, and nipagin.

Moringa Leaf Ethanol Extract Preparation

Moringa leaf simplicia was extracted by the maceration method over three days, with stirring occurring 2–3 times daily. The macerate was collected in a glass bottle each day, and the solvent was replenished. The gathered macerate was concentrated with a rotary evaporator, and the concentrated macerate was subsequently placed in an oven at 50 °C. Determine the yield of the obtained extract.

NLCs *Moringa* Ethanol Extract Preparation

Making NLCs begins by separating the lipid phase and the water phase (Table 1). The lipid phase is melted at 65 °C or 5 °C above the melting point. The aqueous phase is mixed homogeneously. Next, the liquid lipid phase was mixed with the water phase, stirred with a magnetic stirrer, homogenized, and then sonicated. Leave it at room temperature for 1 hour before measurement (Pratiwi et al., 2024).

Table 1. Optimal formula for *Moringa* leaf ethanol extract NLCs

Material	Concentration (%)
Moringa leaf ethanol extract	5
GMS	32
flaxseed oil	32
Tween 80	32
Nipagin	0.3
Propylene glycol	10
Aquadest	ad 100

Making Incision Wounds in White Rats

This study protocol received ethical approval from the Ethics Committee of the Faculty of Medicine, Universitas Tanjungpura, in accordance with the WHO 2011 standards and CIOMS 2016 guidelines. The study was designed in accordance with the 3Rs (Replacement, Reduction, and Refinement) to ensure the ethical use of experimental animals. A total of sixteen male Wistar rats weighing 150–250 g and aged 2–3 months were used. The test animals were acclimatized for 1 week before treatment to help them become accustomed to the experimental conditions and maintain their health. Test animals were anesthetized using ketamine i.m. ketamine was also used as an analgesic (pain reliever) because an incision was made. The hair of the test animal was shaved using a razor on the back and cleaned using 70% alcohol. A 2 cm-long, 2 mm-deep wound was made on the rat's back using a sterile scapel number 1 (Ginting et al., 2022; Megawati et al., 2020). Post-incision, test animals are placed in individual cages for hygiene, infection control, and observation of recovery. Euthanasia by cervical dislocation is performed at the end of the experiment to stop the suffering of the test animals. Cervical dislocation is done by skilled personnel with great care to ensure the procedure is done ethically and humanely.

Treatment of Cut Wounds in White Rats

Normal control group, which was not given treatment; positive control group, which was given betadine ointment; negative control, which was treated with NLCs base without active substances; Experimental group, which was treated using the formula optimum NLCs *Moringa* leaf extract and gel. Treatment and observation of NLCS were performed for 14 days, with 0.3 g smeared twice daily (morning and evening). Observations were made by calculating changes in wound area in each group of test animals to obtain data on the percentage of wound healing (Liana & Utama, 2018).

Data Analysis

Incision wounds on test animals were photographed with a digital camera. Each photo was quantified using the parameter of the area of the incision wound. Quantification was assisted by the Macbiophotonics Image J computer program to obtain the results of the area of the incision wound. Macbiophotonics image J is a program/software that can be used to quantify the area, number, and intensity of an observed research object. Next, a number will be obtained that can be quantified and analyzed. The research wound area data were then analyzed statistically using SPSS.

RESULTS

The findings of this study demonstrate the formulation characteristics and biological activity of nanostructured lipid carriers (NLCs) containing *Moringa* leaf ethanolic extract. The results are organized into three sections: (1) characterization of the NLC formulation (Figure 1), (2) evaluation of the wound-healing process (Figure 2) based on wound closure observations, and (3) statistical analysis of wound closure outcomes using Tukey's HSD test (Figures 3 & 4) to identify significant differences between treatment groups.

Figure 1. NLCs of *Moringa* leaf extract ethanol preparation

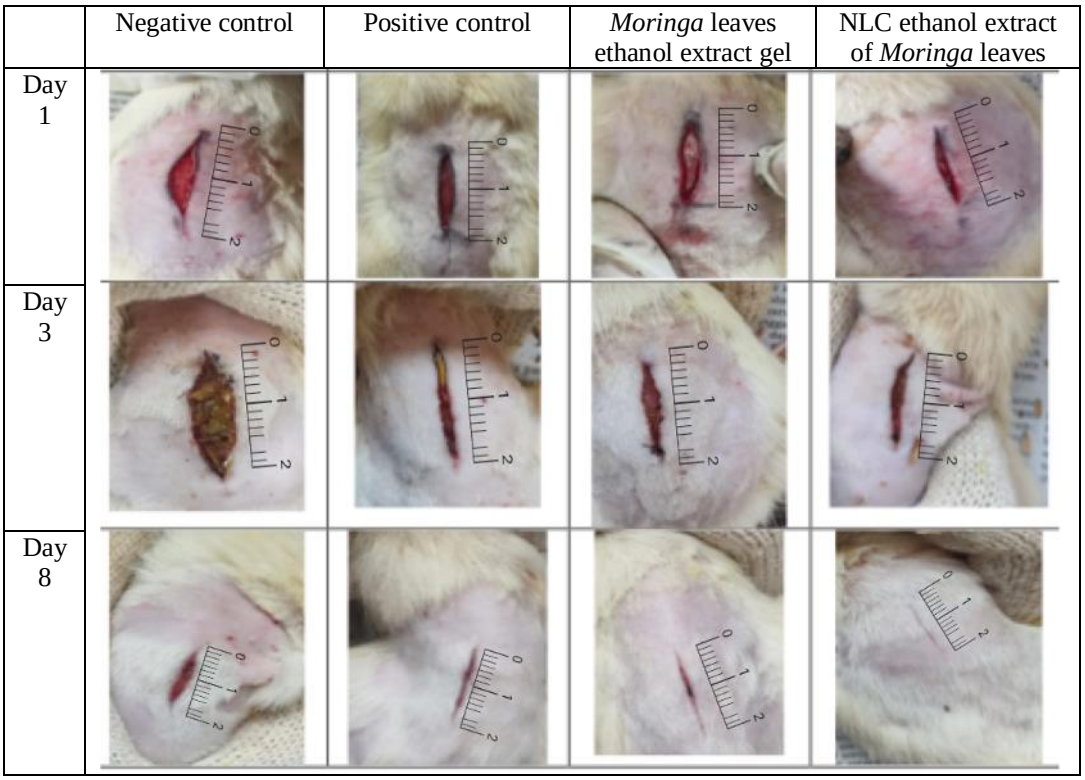

Figure 2. Wound healing process

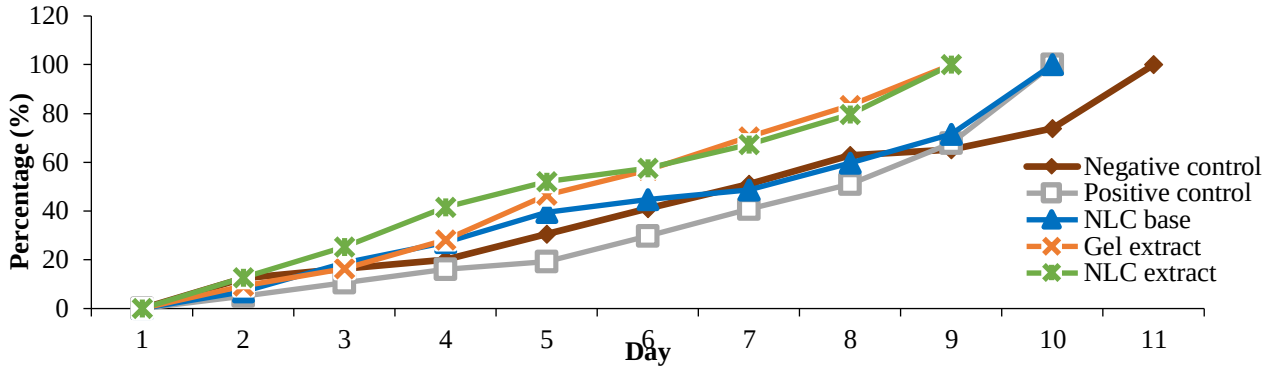

Figure 3. Percentage wound closure

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Kelompok Uji	(J) Kelompok Uji	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Hari Ke-0	Kelompok Negatif	Kelompok Positif	-.11000	.09037	.742	-.3891	.1691
		Kelompok Basis	-.06250	.09037	.955	-.3416	.2166
		Kelompok Gel Ekstrak	-.03750	.09037	.993	-.3166	.2416
		Kelompok NLC Ekstrak	-.11750	.09037	.695	-.3966	.1616
	Kelompok Positif	Kelompok Negatif	.11000	.09037	.742	-.1691	.3891
		Kelompok Basis	.04750	.09037	.983	-.2316	.3266
		Kelompok Gel Ekstrak	.07250	.09037	.926	-.2066	.3516
		Kelompok NLC Ekstrak	-.00750	.09037	1.000	-.2866	.2716
	Kelompok Basis	Kelompok Negatif	.06250	.09037	.955	-.2166	.3416
		Kelompok Positif	-.04750	.09037	.983	-.3266	.2316
		Kelompok Gel Ekstrak	.02500	.09037	.999	-.2541	.3041
		Kelompok NLC Ekstrak	-.05500	.09037	.972	-.3341	.2241
	Kelompok Gel Ekstrak	Kelompok Negatif	.03750	.09037	.993	-.2416	.3166
		Kelompok Positif	-.07250	.09037	.926	-.3516	.2066
		Kelompok Basis	-.02500	.09037	.999	-.3041	.2541
		Kelompok NLC Ekstrak	-.08000	.09037	.898	-.3591	.1991
	Kelompok NLC Ekstrak	Kelompok Negatif	.11750	.09037	.695	-.1616	.3966
		Kelompok Positif	.00750	.09037	1.000	-.2716	.2866
		Kelompok Basis	.05500	.09037	.972	-.2241	.3341
		Kelompok Gel Ekstrak	.08000	.09037	.898	-.1991	.3591
Hari Ke-3	Kelompok Negatif	Kelompok Positif	1.88750	3.22714	.975	-8.0777	11.8527
		Kelompok Basis	-1.14750	3.22714	.996	-11.1127	8.8177
		Kelompok Gel Ekstrak	-9.16750	3.22714	.079	-19.1327	.7977
		Kelompok NLC Ekstrak	-10.00250*	3.22714	.049	-19.9677	-.0373
	Kelompok Positif	Kelompok Negatif	-1.88750	3.22714	.975	-11.8527	8.0777
		Kelompok Basis	-3.03500	3.22714	.877	-13.0002	6.9302
		Kelompok Gel Ekstrak	-11.05500*	3.22714	.026	-21.0202	-1.0898
		Kelompok NLC Ekstrak	-11.89000*	3.22714	.016	-21.8552	-1.9248
	Kelompok Basis	Kelompok Negatif	1.14750	3.22714	.996	-8.8177	11.1127
		Kelompok Positif	3.03500	3.22714	.877	-6.9302	13.0002
		Kelompok Gel Ekstrak	-8.02000	3.22714	.146	-17.9852	1.9452
		Kelompok NLC Ekstrak	-8.85500	3.22714	.094	-18.8202	1.1102
	Kelompok Gel Ekstrak	Kelompok Negatif	9.16750	3.22714	.079	-.7977	19.1327
		Kelompok Positif	11.05500*	3.22714	.026	1.0898	21.0202
		Kelompok Basis	8.02000	3.22714	.146	-1.9452	17.9852
		Kelompok NLC Ekstrak	-.83500	3.22714	.999	-10.8002	9.1302
	Kelompok NLC Ekstrak	Kelompok Negatif	10.00250*	3.22714	.049	.0373	19.9677
		Kelompok Positif	11.89000*	3.22714	.016	1.9248	21.8552
		Kelompok Basis	8.85500	3.22714	.094	-1.1102	18.8202
		Kelompok Gel Ekstrak	.83500	3.22714	.999	-9.1302	10.8002
Hari Ke-8	Kelompok Negatif	Kelompok Positif	.77250	2.03387	.995	-5.5079	7.0529
		Kelompok Basis	-.72500	2.03387	.996	-7.0054	5.5554
		Kelompok Gel Ekstrak	-27.21750*	2.03387	.000	-33.4979	-20.9371
		Kelompok NLC Ekstrak	-27.21750*	2.03387	.000	-33.4979	-20.9371
	Kelompok Positif	Kelompok Negatif	-.77250	2.03387	.995	-7.0529	5.5079
		Kelompok Basis	-1.49750	2.03387	.944	-7.7779	4.7829
		Kelompok Gel Ekstrak	-27.99000*	2.03387	.000	-34.2704	-21.7096
		Kelompok NLC Ekstrak	-27.99000*	2.03387	.000	-34.2704	-21.7096
	Kelompok Basis	Kelompok Negatif	.72500	2.03387	.996	-5.5554	7.0054
		Kelompok Positif	1.49750	2.03387	.944	-4.7829	7.7779
		Kelompok Gel Ekstrak	-26.49250*	2.03387	.000	-32.7729	-20.2121
		Kelompok NLC Ekstrak	-26.49250*	2.03387	.000	-32.7729	-20.2121
	Kelompok Gel Ekstrak	Kelompok Negatif	27.21750*	2.03387	.000	20.9371	33.4979
		Kelompok Positif	27.99000*	2.03387	.000	21.7096	34.2704
		Kelompok Basis	26.49250*	2.03387	.000	20.2121	32.7729
		Kelompok NLC Ekstrak	.00000	2.03387	1.000	-6.2804	6.2804
	Kelompok NLC Ekstrak	Kelompok Negatif	27.21750*	2.03387	.000	20.9371	33.4979
		Kelompok Positif	27.99000*	2.03387	.000	21.7096	34.2704
		Kelompok Basis	26.49250*	2.03387	.000	20.2121	32.7729
		Kelompok Gel Ekstrak	.00000	2.03387	1.000	-6.2804	6.2804

*. The mean difference is significant at the 0.05 level.

Figure 4. Statistical analysis of wound closure ability with Tukey HSD test

DISCUSSION

This research aims to enhance wound-healing efficacy in white rats (*Rattus norvegicus*) using NLCs containing an ethanol extract of *Moringa oleifera* leaves. *M. oleifera* leaves are extracted to obtain extracts with active compounds that have various effects. Ethanol extract of *Moringa oleifera* leaves. *M. oleifera* leaves are shown in Figure 1, including wound healing. The present study demonstrated that both the *Moringa* leaf ethanolic extract gel and the NLC-loaded *Moringa* extract accelerated wound healing compared with the positive and negative control groups. Notably, complete wound closure (100%) was achieved on day 8 in the extract-treated groups, whereas the positive control required 10 days and the negative control required 14 days to achieve complete closure. These findings indicate that *Moringa* leaf extract exhibits significant wound-healing activity and that its incorporation into an NLC system may further enhance its therapeutic efficacy.

The accelerated wound-healing effect observed in the NLC-loaded *Moringa* extract can be explained by the synergistic interaction between the pharmacological activity of *Moringa* bioactive compounds and the technological advantages of the NLC delivery system. Flavonoids, polyphenols, and saponins present in *Moringa* leaves are known to suppress inflammatory mediators through inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) pathways, thereby reducing excessive inflammation during the early stage of wound healing. In addition, these compounds stimulate fibroblast proliferation, collagen synthesis, and extracellular matrix remodeling, which are essential for tissue regeneration. Simultaneously, the lipid matrix of NLCs enhances skin hydration through occlusive effects, reduces transepidermal water loss, and improves the penetration and retention of active compounds within the skin layers. This combination creates a favorable microenvironment for wound repair, resulting in faster wound contraction and closure.

The findings of the present study are consistent with previous reports demonstrating the wound-healing potential of *M. oleifera* extracts and the ability of lipid-based nanocarriers to enhance topical drug delivery. Several studies have reported that *Moringa*-derived phytochemicals promote fibroblast activity, collagen deposition, and anti-inflammatory responses. At the same time, NLC formulations enhance dermal bioavailability and prolong the residence time of active compounds at the target site. The faster wound closure observed in the present study supports these previous observations and suggests that NLC-mediated delivery may optimize the therapeutic benefits of *Moringa* leaf extract.

Nevertheless, this study has several limitations. The number of animals per group was relatively small because the study was designed according to the reduction principle of the 3R framework. Furthermore, the wound-healing assessment was limited to macroscopic observations of wound closure, without histopathological evaluation, collagen quantification, analysis of inflammatory biomarkers, or skin penetration studies. Therefore, further investigations involving larger sample sizes and mechanistic evaluations are required to confirm the biological pathways underlying the observed wound-healing effects.

In this study, *Moringa* leaf simplicia was extracted using the maceration method with an ethanol solvent for 3 days. The collected macerate was then extracted using a rotary evaporator and dried in an oven. This method is designed to yield a concentrated extract suitable for formulating nanostructured lipid carriers (NLCs) for topical applications. *Moringa* leaves (*Moringa oleifera*) contain various bioactive compounds that can support wound healing, including antioxidants, antimicrobials, and anti-inflammatories. Numerous studies indicate that Moringa leaf extract has active components, including flavonoids, polyphenols, saponins, and ascorbic acid, which can promote tissue regeneration and diminish inflammation in the wound vicinity (Rowe et al., 2009). Compounds in *Moringa* leaves, such as flavonoids and polyphenols, possess anti-inflammatory activities by inhibiting the enzymes cyclooxygenase (COX) and lipoxygenase (LOX), which are responsible for the synthesis of prostaglandins that promote inflammation. Flavonoids and polyphenols can speed up wound healing by reducing swelling and pain in the wound area. *M. oleifera* also has strong antimicrobial properties, which help prevent secondary infections in wounds. This compound inhibits the growth of pathogenic bacteria, which can slow down the wound-healing process. Several studies show that *Moringa* leaf extract can increase fibroblast cell proliferation and stimulate collagen synthesis in wound tissue, which is vital in wound healing (Ermawati et al., 2024).

NLCs are lipid-based drug delivery systems that have the advantage of increasing the stability and bioavailability of active ingredients. NLCs have a denser structure than traditional liposomes. They can better protect the active ingredients from external environmental factors (such as heat and oxygen) and allow their gradual release (Elmowafy & Al-Sanea, 2021). Studies indicate that NLC formulations can enhance the absorption of active substances into the skin and wound tissue. This is due to the small particle size of NLCs (usually 50–200 nm), which allows them to pass through the stratum corneum of the skin and reach the epidermis and dermis more efficiently. In addition, the gradual release properties of NLCs allow *Moringa* leaf extract to provide a more prolonged therapeutic effect on wounds, improving healing by reducing the frequency of application and maintaining an adequate concentration of active ingredients in the wound area for a longer period.

The NLC formula for *Moringa* leaf extract consists of solid lipids, liquid lipids, and surfactants. The lipid phase, in its liquid and solid forms, produces NLCs with distinct characteristics when combined with active substances. The choice of lipid type is often determined by the solubility of the active component or drug in the lipid material (Zhang et al., 2017). Solid lipids, as the main components in NLC synthesis, facilitate the formation of solid particles, enhance loading capacity, and influence the stability of the active substances (Garcês et al., 2018; Muller et al., 2007; Zhang et al., 2017). Liquid lipids can alter the regularity of the crystal lattice of a solid lipid matrix, resulting in an irregular lattice and increasing the capacity for active chemicals (Ebtavanny et al., 2018). The solid lipid used to dissolve *Moringa* leaf extract is glyceryl monostearate (GMS). GMS can work effectively as a solvent and stabilizer by dissolving polar and nonpolar components as an emulsion. GMS has an HLB value of 3.8 with good solubility in mineral oil. This property has the benefit of being a dispersing agent for the lipid phase to dissolve active substances (Rowe et al., 2009). Glyceryl monostearate (GMS) is an amphiphilic lipid containing a polar glycerol moiety with hydroxyl groups and a nonpolar stearate fatty acid chain. This structural characteristic enables. GMS acts as an effective emulsifier and stabilizer in nanostructured lipid carrier formulations (Ammar et al., 2016). In research on NLCs of honey extracts, results showed that the solid lipid capable of dissolving the highest amount of extract was GMS (Pratiwi et al., 2024). *Flaxseed oil* is a functional vegetable oil that contains high levels of α-linoleic acid. Flaxseed oil is often combined with other oils because it can form polymers (Huang et al., 2017)

Surfactants used as emulsifying agents in NLC formulations significantly affect the dispersion and stability of the preparation (Zhang et al., 2017). Surfactants reduce the interfacial tension between the lipid mixture and the dispersing agent (water), thereby inhibiting particle aggregation and maintaining particle size (Muller et al., 2007). Nonionic surfactants are surfactants characterized by uncharged alkyl groups. Nonionic surfactants are safer, compatible with the skin, and show quality and minimal skin irritation when applied (Lukic et al., 2016). This research uses Tween 80, an amphiphilic compound and hydrophilic nonionic surfactant, capable of producing stable, soluble oil-in-water emulsions. In addition, there is a decrease in interfacial tension, thereby reducing the likelihood of separation between the oil/water phases (Rowe et al., 2009). This study used 10% propylene glycol as a cosurfactant in the NLC formulation (Pratiwi et al., 2024).

The results showed that the *Moringa* leaf ethanol extract gel group and *Moringa* leaf ethanol extract NLCs could heal cut wounds more quickly than the drug control and negative control groups, with wound closure reaching 100% on the eighth day. In contrast, the drug control group took until the 10th day, while the negative control group only achieved 100% wound closure on the 14th day. The Wound healing process is shown in Figure 2. he faster rate of wound healing in the *Moringa* leaf ethanol extract gel and NLCs treatment group was influenced by the presence of bioactive compounds in *Moringa* leaves, which support the process of cell proliferation and collagen formation, as well as anti-inflammatory effects, which reduce swelling and pain. Although both the conventional gel and NLC formulations achieved complete wound closure by day 8, the development of the NLC system remains scientifically relevant. NLCs have been reported to improve the stability of encapsulated phytochemicals, enhance skin hydration through occlusive effects, increase residence time at the application site, and provide sustained release of active compounds. These pharmaceutical benefits may not be fully reflected in wound closure measurements alone during the relatively short

observation period of this study. Therefore, further investigations into release kinetics, skin permeation, stability testing, and chronic wound models are required to determine whether the NLC formulation offers additional therapeutic benefits beyond those observed in the present study (Alquraisy et al., 2026). The drug control showed slower wound closure (100% on day 10), which may be due to its lower bioavailability and stability compared with the NLC-based formulation. While the negative control group, which received no treatment or placebo ointment, had the slowest wound closure (100% on day 14) as shown in Figure 3, the active intervention accelerated wound healing. The effectiveness of wound closure in this study is shown in Figures 2 and 3.

At the outset (day 0), no significant difference was observed between the groups, indicating that their initial conditions were comparable. On day 3, significant differences appeared between the extract groups (gel and NLC) and the positive control. Furthermore, on day 8, the extract group showed significant differences against all controls (negative, positive, and base), but not between the gel and NLC. The result confirms that the extract is effective, with the most pronounced effects observed on day 8. The statistical analysis of wound closure ability with the Tukey HSD test is shown in Figure 4.

The present findings are consistent with previous reports demonstrating the wound-healing activity of *Moringa oleifera* extracts. Several studies have shown that *Moringa*-derived flavonoids, polyphenols, and saponins accelerate tissue regeneration by reducing inflammation, promoting fibroblast proliferation, and enhancing collagen deposition. Similarly, previous investigations on lipid-based nanocarrier systems have reported improved dermal delivery, enhanced skin hydration, and prolonged retention of bioactive compounds at the wound site. The comparable wound closure observed between the gel and NLC formulations in the present study suggests that the pharmacological activity of *Moringa* extract plays a dominant role in acute wound healing, whereas the additional benefits of NLC technology may require further evaluation using more sensitive pharmaceutical and biological endpoints (Montenegro, 2017; Singh et al., 2024)

The results of this study indicate that the *Moringa* leaf extract NLC formulation can be used in wound-healing therapy, both for minor and chronic wounds. The advantages of NLC formulations in enhancing penetration and gradual release of active ingredients make them an excellent choice compared to conventional topical preparations. The results of the present study suggest that NLC-loaded *Moringa* leaf extract may be a promising topical formulation for accelerating wound healing in an acute wound model. However, because this study was conducted in healthy animals with acute incision wounds, the potential efficacy of this formulation in chronic wounds, including diabetic wounds, remains speculative and should be evaluated in future studies using appropriate disease-specific animal models. This research also shows that *Moringa* leaf extract can accelerate wound healing and reduce inflammation through tissue regeneration.

CONCLUSION

Moringa leaf ethanol extract NLC preparations have the potential as an effective topical therapy in accelerating wound healing. The advantages of NLCs in increasing the penetration of active ingredients and providing gradual release are the main factors differentiating this preparation from the control group of conventional drugs. The treatment group of *Moringa* leaf ethanol extract gel and *Moringa* leaf ethanol extract NLCs could heal cuts faster than the drug control and negative control groups, with wound closure reaching 100% on day 8. The preparation of *Moringa* leaf ethanol extract NLCs has the potential to be an effective topical therapy in accelerating wound healing.

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