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Antioxidant, antimicrobial, and wound-healing effects of *Moringa oleifera* extract on incised wounds in rats

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ABSTRACT: This study aims to evaluate the potential of *Moringa oleifera* extracts in accelerating incisional wound healing in rats, with a focus on antimicrobial activity and the modulation of wound-healing-related gene expression. Incisional wounds infected by microorganisms remain a significant health problem, particularly in tropical countries such as Indonesia. Extracts derived from the roots, stems, and leaves of *Moringa oleifera* contain various bioactive compounds known for their antimicrobial and therapeutic properties. Extraction was performed separately on the roots, stems, and leaves. Antibacterial activity of all extracts was evaluated against *Staphylococcus aureus*. Among the tested plant parts, the root extract demonstrated the strongest antibacterial activity and was therefore selected for further analysis. Gas chromatography–mass spectrometry (GC/MS) was conducted on the root extract to identify bioactive compounds responsible for its antimicrobial effects. An in vivo study using a rat incisional wound model was subsequently performed to evaluate the wound-healing efficacy of the root extract. Gene expression analysis revealed increased expression of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), along with a decreased expression of transforming growth factor beta (TGF-β) in treatment groups compared to controls. These findings suggest that *Moringa oleifera* root extract has strong antibacterial properties and can positively influence wound-healing-related gene expression, supporting its potential development as an effective and affordable wound-healing ointment for use in tropical regions.

1. INTRODUCTION

Incisional wounds are a type of open wound that are highly susceptible to microbial infections such as bacteria, viruses, and fungi. These infections not only impede the healing process but also increase the risk of serious complications, including death. Infections can significantly delay wound healing and contribute to high morbidity rates (Ding et al., 2022). In Indonesia, the challenges of managing wound infections are exacerbated by environmental factors such as a tropical climate, suboptimal sanitation, and high population density (Ministry of Health of Indonesia, 2021). Therefore, effective and safe treatment methods are crucial to prevent infections and accelerate wound healing.

Moringa oleifera, commonly known as moringa, is widely recognized for its nutritional content and bioactive compounds that benefit human health. Various studies have shown that moringa is rich in antioxidants, vitamins,

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minerals, and phenolic compounds that provide positive health effects. Specifically, moringa extracts have been proven to possess strong antimicrobial activity against wound infection pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Anwar et al., 2007; Royani et al., 2023). In addition, active compounds in moringa, including flavonoids and phenolic acids, have been shown to enhance the activity of the body's antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), through the activation of the Nrf2 pathway (Mundkar et al., 2022).

The wound healing process involves a series of complex biological stages, including inflammation, proliferation, and remodeling of tissues. At each stage, the role of antioxidant enzymes is crucial for reducing oxidative stress and maintaining redox balance within cells (Wang & Graves, 2020). High oxidative stress can significantly delay the wound healing process and exacerbate inflammation, leading to more serious complications. Previous research indicates that the administration of antioxidants can accelerate wound healing by reducing oxidative damage to the cells involved in tissue regeneration.

Moringa oleifera not only serves as a source of antioxidants but also has the potential to enhance the expression of genes related to wound healing, such as vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF-β). These compounds play a vital role in the processes of angiogenesis and tissue regeneration, which are essential for effective wound healing (Parihar et al., 2023). Therefore, utilizing moringa as an adjunct therapy in wound healing presents an intriguing area for further research.

Prior studies have demonstrated that moringa extracts can enhance antioxidant activity and exhibit significant antimicrobial effects. In laboratory tests, moringa extracts have been shown to effectively inhibit the growth of various pathogenic bacteria (Anwar et al., 2007). This suggests that moringa could be a promising alternative in managing wound infections, especially in areas with limited access to modern medical care.

This study aims to evaluate the effectiveness of extracts from the leaves, stems, and roots of *Moringa oleifera* on the healing of incisional wounds in a rat model. Furthermore, this research will analyze the antioxidant and antimicrobial activities of the extracts, as well as their effects on the expression of genes involved in the wound healing process. Thus, the results are expected to contribute to the development of herbal therapies based on local resources that are effective and affordable in tropical regions.

Through this research, it is hoped that an optimal formulation of moringa extract can be developed for use as an ointment or topical product for wound healing. By leveraging local resources, this study has the potential not only to improve public health but also to open avenues for the development of economical and environmentally friendly herbal products.

Finally, this research aims to provide new insights into the potential of *Moringa oleifera* in the fields of pharmacology and wound healing therapy, as well as to encourage further studies in herbal medicine and evidence-based traditional healing practices.

2. MATERIAL AND METHODS

2.1. Materials

Plant materials used in this study consisted of *Moringa oleifera* roots, stems, and leaves. Microbiological materials included *S. aureus* and Mueller–Hinton Agar (MHA). Sodium carboxymethyl cellulose (Na-CMC) was used as the negative control, while tetracycline was used as the positive control. Male Wistar rats were used as experimental animals and were provided with standard feed and water ad libitum. Primers specific for SOD, CAT, and TGF-β were used for gene expression analysis.

2.2. Plant material

Moringa oleifera leaves, stems, and roots were collected from Kabupaten Sidenreng Rappang, Indonesia. After collection, the materials were washed, air-dried, and ground into a fine powder for extraction.

2.3. Extraction of *Moringa oleifera*

The extraction process involved macerating 100 g of dried Moringa powder in 500 mL of ethanol for 72 hours. The mixture was stirred continuously and filtered through Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator at 40°C to obtain dry extracts, which were stored at 4°C until further use.

2.4. Antimicrobial activity testing

The antimicrobial activity of Moringa extracts was evaluated against *S. aureus* using the disk diffusion method. Bacterial cultures were prepared and spread uniformly on MHA plates. Disks impregnated with different concentrations of the extracts (0.5%, 2%, 4%, and 6%) were placed on the agar surface. The plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured in millimeters.

2.5. Gas Chromatography–mass spectrometry (GC-MS) analysis

GC/MS analysis was performed to identify the chemical constituents of the *Moringa oleifera* extract that exhibited the strongest antimicrobial activity. The analysis was conducted using a GC/MS instrument equipped with a capillary column and helium as the carrier gas. The injector and detector temperatures were set according to standard operating conditions.

The mass spectra of the detected compounds were identified by comparing their fragmentation patterns with those in standard mass spectral libraries.

2.6. Wound healing studies

The wound healing efficacy of Moringa extracts was assessed using a rat model. A total of 35 male Wistar rats were used in the study. Wounds were created on the dorsal side of the rats, and the animals were divided into groups receiving different treatments: control (no treatment), Moringa extract (5% and 10%), and a standard treatment (mupirocin). The treatments were applied topically once daily. Wound contraction was measured on days 3, 6, 9, 12, and 15 by calculating the percentage of wound area reduction. The percentage of wound contraction was determined using the formula:

$$\text{Wound contraction (\%)} = \frac{\text{Initial wound area} - \text{Final wound area}}{\text{Initial wound area}} \times 100$$

On day 15, healed wound tissue samples were collected for analysis of gene expression.

2.7. Gene expression analysis

The expression of antioxidant genes (SOD, CAT) and healing-related genes (TGF-β) was analyzed using quantitative PCR (qPCR). Total RNA was extracted from the wound tissue samples collected on day 15, and cDNA was synthesized. Gene expression levels were quantified using specific primers, and the results were normalized to a housekeeping gene.

2.8. Statistical analysis

Data were analyzed using SPSS version 22. The results were expressed as mean ± standard deviation (SD). Differences between groups were evaluated using ANOVA, followed by post hoc tests. A p-value of <0.05 was considered statistically significant.

2.9. Ethical considerations

The study was conducted in accordance with ethical guidelines for animal research. Approval for the study was obtained from the Ethics Committee of the Faculty of Medicine, Hasanuddin University, under protocol number UH25040228, with the formal approval number 245/UN4.6.4.5.31/PP36/2025. The research was also endorsed by the Research and Development Center (RSPTN) of Hasanuddin University and RSUP Dr. Wahidin Sudirohusodo Makassar. All animal procedures were performed humanely, ensuring minimal discomfort and pain in accordance with ethical standards.

3. RESULT AND DISCUSSION

Moringa oleifera, commonly known as drumstick tree or moringa, has long been praised for its various health and nutritional benefits. This study aimed to evaluate the potential of *Moringa oleifera* extracts, particularly in the context of antioxidant activity, antimicrobial properties, and wound healing capabilities. The results obtained indicate that Moringa not only possesses strong therapeutic properties but also offers a natural solution to increasing health challenges, such as antibiotic resistance and slow wound healing (Hamid et al., 2025).

Figure 1 shows the ethanolic extracts of *Moringa oleifera* obtained from different plant parts, namely (a) leaves, (b) roots, and (c) stems. Variations in color, clarity, and texture were observed among the extracts, indicating differences in the chemical composition and concentration of secondary metabolites in each plant part. These variations are commonly related to differences in phytochemical profiles, including isothiocyanates, phenolic compounds, flavonoids, and other bioactive constituents (El-Saadony et al., 2025).

Previous studies have reported that *Moringa oleifera* contains a wide range of bioactive compounds with significant biological activities. Isothiocyanates derived from Moringa have been shown to possess diverse pharmacological effects, including antimicrobial and metabolic regulatory activities, which may contribute to their therapeutic potential (Hamid et al., 2025). Differences in extraction outcomes among plant parts are influenced by the distribution of these compounds, which is known to vary between leaves, roots, and stems.

Ethanolic extraction is effective in isolating polar and semi-polar compounds that are responsible for various biological activities. Acid-assisted and ethanol-based extraction methods have been reported to enhance the recovery of bioactive compounds from Moringa, particularly those contributing to health-promoting effects (Ahsan et al., 2025). Furthermore,

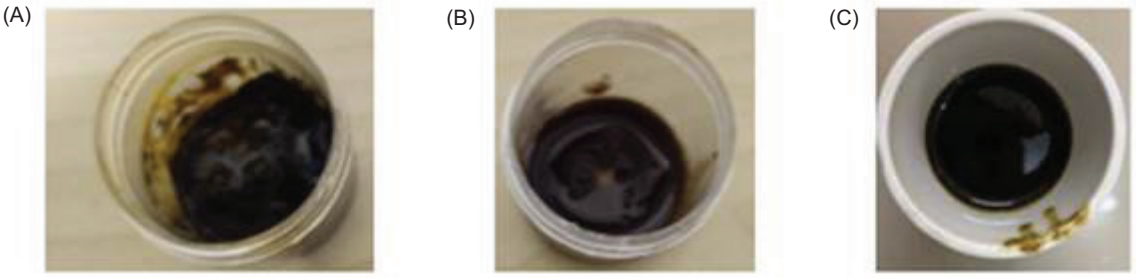

Figure 1. Ethanolic extracts of Moringa oleifera. (A) leaf, (B) root, and (C) stem.

bioactive constituents from Moringa have demonstrated strong antioxidant-related biological activities, supporting their potential role in tissue repair and protection against oxidative stress (Hardjo et al., 2025).

The observable differences among the ethanolic extracts in Figure 1 support the premise that each plant part possesses a distinct phytochemical profile, which may influence antimicrobial efficacy. These findings justify subsequent antibacterial screening to identify the extract with the strongest antimicrobial activity for further GC/MS characterization and in vivo wound-healing evaluation.

Figure 2 presents the antioxidant activity (IC_{50}) of ethanolic extracts obtained from the leaves, stems, and roots of Moringa oleifera. Among the tested extracts, the root extract exhibited the strongest antioxidant activity, as indicated by the lowest IC_{50} value, followed by the stem and leaf extracts. These findings suggest that the root contains higher concentrations of antioxidant phytochemicals, supporting its selection for further antimicrobial and wound-healing investigations. Table 1 summarizes the antibacterial activity of the ethanolic extracts of Moringa oleifera against Staphylococcus aureus. The root extract produced significantly larger inhibition zones than the stem and leaf extracts at all tested concentrations, with the strongest antibacterial activity observed at 4% and 6% concentrations. These findings indicate that the root extract possesses superior antibacterial efficacy and was therefore selected for subsequent GC–MS characterization and in vivo wound-healing evaluation (Asfaw et al., 2023).

Moringa extracts have demonstrated significant antimicrobial efficacy, particularly against *S. aureus*, a common pathogen in wound infections. The root extract showed the most potent antibacterial activity at concentrations of 4% and 6%, making it a promising candidate for further research in wound healing applications (Parihar et al., 2023). This finding underscores *Moringa oleifera*' *s* potential as a natural alternative to conventional antibiotics, especially in areas with limited access to medical care.

Among the active compounds identified, palmitoleic acid plays a crucial role due to its antimicrobial properties. It disrupts bacterial cell membranes, leading to increased permeability and cell lysis, while also inhibiting essential metabolic pathways that prevent bacterial growth. This multifaceted action enhances the overall antibacterial efficacy of Moringa extracts, highlighting their relevance in addressing the global challenge of antibiotic resistance. Moreover, the synergistic effects of various bioactive components in Moringa, including flavonoids and phenolic acids, further contribute to its therapeutic potential. These compounds not only bolster the antimicrobial effects but also provide antioxidant and anti-inflammatory benefits, which are critical for effective wound healing (Ahsan et al., 2025). By reducing oxidative stress and inflammation, *Moringa oleifera* presents a comprehensive strategy for wound management, emphasizing the need for further investigation into its applications in modern medicine.

Figure 3 shows the antibacterial activity of the ethanolic extracts against Staphylococcus aureus. The root extract produced the largest inhibition zones, particularly at concentrations of 4% and 6%, indicating superior antibacterial efficacy compared with the stem and leaf extracts. These findings are consistent with the quantitative inhibition-zone measurements summarized in Table 1, confirming that the root extract possesses the highest antibacterial potential among the tested plant parts. Based on these results, the root extract was selected for subsequent GC–MS characterization and in vivo wound-healing evaluation.

The identification of bioactive compounds is essential for understanding the antimicrobial and wound-healing potential of *Moringa oleifera*. Plant extracts with strong antibacterial activity often contain a complex mixture of secondary metabolites that act individually or synergistically to inhibit microbial growth and reduce the risk of wound infection. These bioactive compounds may also contribute to wound healing by modulating inflammation, preventing microbial colonization, and supporting tissue repair. GC–MS is a powerful analytical technique widely used for the identification of volatile and semi-volatile compounds in plant extracts and provides valuable information on their chemical composition (Taher et al., 2025).

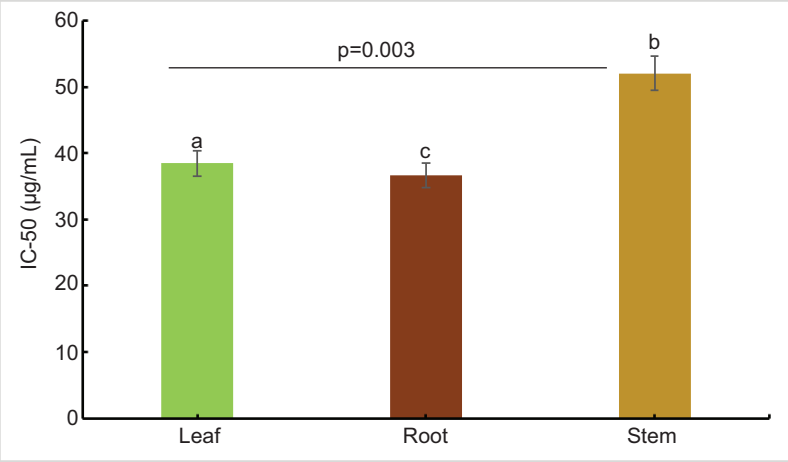

Figure 2. Antioxidant activity (IC_{50} values) of ethanolic extracts of Moringa oleifera root, stem, and leaf using the DPPH radical scavenging assay.
Note: Data were analyzed using one-way ANOVA with a significance level of $p < 0.05$. Post hoc analysis was performed using Tukey's test. Different superscript letters (a, b, etc.) indicate statistically significant differences between groups ($p < 0.05$).

Table 1
Antimicrobial activity of ethanolic extracts of Moringa oleifera root, stem, and leaf against *Staphylococcus aureus.*

Extract	Inhibition zone diameter (mm ± SD)					
	0.50%	**2%**	**4%**	**6%**	**Tetracycline**	**Na-CMC**
Leaf	2.50 ± 0.69[a]	3.50 ± 0.71[a]	5.00 ± 0.78[a]	8.00 ± 0.61[a]	21.50 ± 0.56	00.00 ± 0.00
Root	7.50 ± 0.04[c]	8.50 ± 0.50[c]	9.50 ± 0.76[c]	12.50 ± 0.83[c]	22.00 ± 0.40	00.00 ± 0.00
Stem	4.50 ± 0.87[b]	5.50 ± 0.58[b]	6.50 ± 0.3[b]	9.50 ± 0.49[b]	21.50 ± 0.51	00.00 ± 0.00
p-value	P<0.001	P < 0.001	P < 0.001	P < 0.001	P = 0.422	–

Note: Data were analyzed using one-way ANOVA with a significance level of $p < 0.05$. Post hoc analysis was performed using Tukey's test. Different superscript letters (a, b, etc.) indicate statistically significant differences between groups ($p < 0.05$).

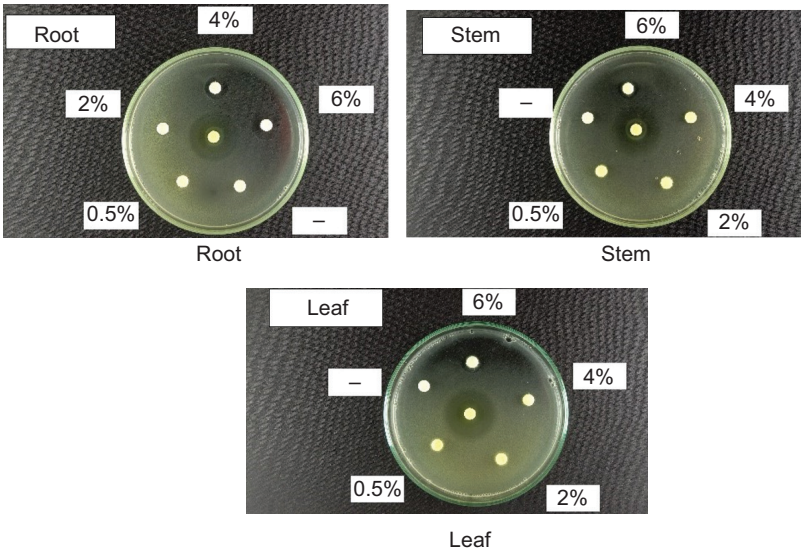

Figure 3. Antibacterial activity of ethanolic extracts of *Moringa oleifera* root, stem, and leaf against *Staphylococcus aureus*.

In this study, GC–MS analysis was employed to characterize the chemical constituents of the *Moringa oleifera* extract that exhibited the strongest antimicrobial activity against *S. aureus*. The identification of these compounds is important for elucidating the possible mechanisms underlying the observed antibacterial effects and for supporting the selection of specific bioactive components that may contribute to wound-healing applications (Anzano et al., 2022).

Figure 4 presents the GC–MS chromatogram of the ethanolic root extract of Moringa oleifera. Multiple chromatographic peaks indicate the presence of diverse phytochemical constituents with varying retention times, reflecting the chemical complexity of the extract. Identification of these peaks revealed several bioactive compounds associated with antioxidant, antimicrobial, anti-inflammatory, and wound-healing activities, as summarized in Table 2.

GC–MS analysis of the *Moringa oleifera* extract with the strongest antimicrobial activity revealed the presence of several bioactive compounds known to possess antibacterial, antioxidant, anti-inflammatory, and wound-healing properties. The diversity of compounds detected suggests that the antimicrobial efficacy of the extract is likely due to synergistic interactions among multiple secondary metabolites rather than the effect of a single compound. Table 2 summarizes the major bioactive compounds identified in the ethanolic root extract of Moringa oleifera by GC–MS analysis, together with their reported biological activities and supporting references. The identified constituents include phenolic compounds, fatty acids, phytosterols, triterpenoids, and antioxidant vitamins, all of which have been reported to possess antimicrobial, antioxidant, anti-inflammatory, and wound-healing properties. These compounds are likely to act synergistically, contributing to the overall biological activity of the root extract (Taher et al., 2025).

Among the identified compounds, 2,4-di-tert-butylphenol is reported to exhibit strong antioxidant, antimicrobial, and anti-inflammatory activities, which are essential in preventing oxidative stress and bacterial colonization at the wound site (Zhao et al., 2020). Fatty acids such as palmitoleic acid, palmitic acid, oleic acid, and stearic acid are known to disrupt bacterial cell membranes, contributing to antibacterial effects. In addition, palmitoleic acid has been reported to act as a pro-wound healing lipid by enhancing tissue regeneration and skin barrier function (Weimann et al., 2018).

Ascorbic acid (and its derivatives) and α-tocopherol are potent antioxidant compounds that play important roles in reducing oxidative damage, promoting collagen synthesis, and enhancing epithelialization during wound healing (Bechara et al., 2022; Comino-Sanz et al., 2021). The presence of these antioxidants supports the observed increase in

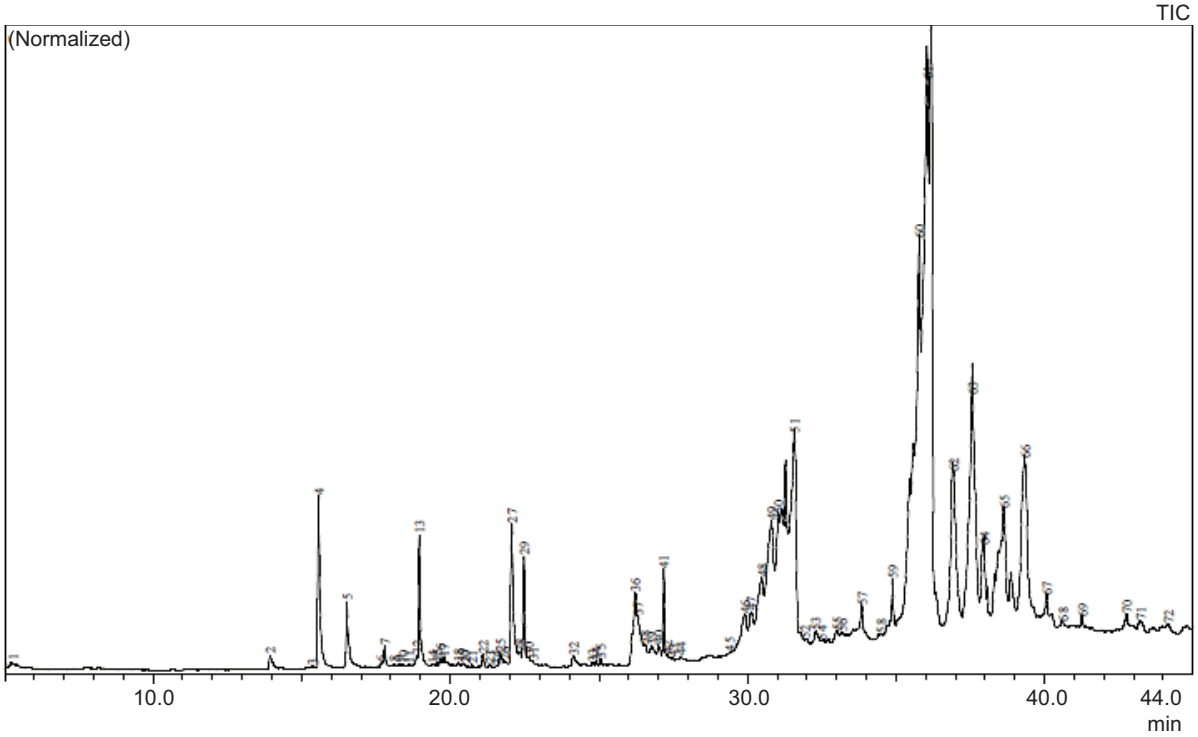

Figure 4. GC–MS chromatogram of the ethanolic root extract of *Moringa oleifera*.

Table 2
Bioactive compounds identified from the ethanolic root extract of *Moringa oleifera* with potential roles in wound healing.

No	Compound name	Peak No.	RT (min)	Relative abundance (%)	Reported biological activity	References
1	2,4-Di-tert-butylphenol	4	15.565	1.35	Antioxidant, antimicrobial, anti-inflammatory	Rouvier et al., 2024
2	Palmitoleic acid (C16:1)	25	21.681	0.18	Antibacterial, pro-wound healing lipid	Weimann et al., 2018
3	Ascorbyl palmitate	23	22.072	1.63	Antioxidant, collagen synthesis enhancer	Sharma et al., 2018
4	Palmitic acid	27	22.072	1.63	Antibacterial, anti-inflammatory	Taher et al., 2025
5	Oleic acid (C18:1)	36	26.225	1.07	Antioxidant, anti-inflammatory, antimicrobial	Pegoraro et al., 2021
6	Stearic acid (C18:0)	39	26.777	0.36	Antibacterial, membrane stabilizer	Dantas et al., 2002
7	γ-Sitosterol	48	30.493	1.87	Anti-inflammatory, antioxidant, antibacterial	Shen et al., 2021
8	β-Sitosterol	55	33.029	0.37	Wound healing, anti-inflammatory	Abbas et al., 2019
9	Lanosterol	60	35.791	20.88	Anti-inflammatory, antioxidant, fibroblast activator	Berger et al., 2004
10	Cycloartenol	65	38.642	6.32	Antioxidant, antibacterial, anti-inflammatory	Wang et al., 2019
11	α-Tocopherol	67	40.089	1.32	Potent antioxidant, enhances epithelialization	Stanescu et al., 2025
12	Methyl oleanolate	72	44.171	0.60	Anti-inflammatory, antioxidant, antibacterial	Jannus et al., 2024

antioxidant-related gene expression and highlights their contribution to tissue repair processes.

Phytosterols such as γ-sitosterol and β-sitosterol have been widely reported to possess anti-inflammatory, antimicrobial, antioxidant, and wound-healing properties by modulating inflammatory responses and promoting fibroblast proliferation and tissue regeneration (Babu & Jayaraman, 2020). Notably, lanosterol and cycloartenol were detected in relatively high abundance, particularly lanosterol, which is known for its anti-inflammatory and antioxidant properties as well as its ability to activate fibroblasts, thereby accelerating tissue regeneration (Sharma, et al., 2021).

Methyl oleanolate, a triterpenoid compound identified in the extract, has also been reported to exhibit anti-inflammatory, antioxidant, and antibacterial activities, further contributing to the overall wound-healing potential of the extract (Pan et al., 2024). Collectively, the presence of these compounds explains the strong antibacterial activity against *S. aureus* and supports the potential application of Moringa oleifera extract as a natural therapeutic agent for wound management.

Wound healing is a complex biological process involving inflammation, tissue proliferation, and remodeling, all of which are highly susceptible to microbial infection and oxidative stress. Infected wounds often experience delayed healing due to excessive inflammation and impaired cellular responses. Therefore, effective wound-healing therapies should not only promote tissue regeneration but also possess antimicrobial and antioxidant properties to support optimal healing In vivo wound-healing models are widely used to evaluate the therapeutic potential of bioactive compounds under physiological conditions. In this study, an in vivo incisional wound model in rats was employed to assess the wound-healing efficacy of *Moringa oleifera* extract with the strongest antimicrobial activity. The evaluation focused on wound closure and the modulation of healing-related gene expression, providing insight into the potential of *Moringa oleifera* as a natural wound-healing agent (Shen et al., 2025).

Figure 4 illustrates the percentage of wound contraction in rats treated with *Moringa oleifera* root extract ointment compared with control groups over a 15-day observation period. All treatment groups showed a progressive increase in wound contraction over time; however, rats treated with *Moringa oleifera* extract, particularly at a concentration of 10%, exhibited a markedly faster wound closure compared to the untreated control group. The wound contraction pattern of the 10% extract group approached that of the positive control (mupirocin), indicating a strong wound-healing efficacy.

The enhanced wound contraction observed in the *Moringa oleifera*–treated groups can be attributed to the presence of bioactive compounds identified in the GC–MS analysis. Compounds such as β-sitosterol and oleanolic acid are well recognized for their anti-inflammatory and tissue-regenerative properties. β-Sitosterol plays a crucial role in reducing excessive inflammation at the wound site, which is essential for effective wound healing, as prolonged inflammation can delay the transition to the proliferative phase and impair tissue repair. In addition, β-sitosterol contributes to cell membrane stabilization, helping to maintain cellular integrity during the healing process. Oleanolic acid has been reported to promote wound healing by enhancing fibroblast proliferation and stimulating collagen synthesis, thereby accelerating tissue regeneration and wound closure (Moyo et al., 2019). The presence of these compounds provides a mechanistic

explanation for the improved wound contraction observed in the *Moringa oleifera*–treated groups, particularly at higher extract concentrations.

Quantitative assessment of wound contraction over 15 days demonstrated that the 10% *Moringa oleifera* extract group exhibited a significantly higher percentage of wound contraction compared to the control group, indicating a more rapid healing response. This accelerated healing process is likely the result of synergistic effects between the antioxidant and antimicrobial properties of the extract. Antioxidant compounds reduce oxidative stress at the wound site, thereby minimizing cellular damage and creating a favorable environment for tissue repair. Simultaneously, the antimicrobial activity of *Moringa oleifera* extracts helps prevent bacterial infection, a major factor that can delay wound healing (Wang et al., 2020).

Furthermore, the wound-healing efficacy of *Moringa oleifera* extract may also be associated with its ability to modulate key molecular pathways involved in inflammation and tissue repair. By influencing the expression of cytokines and growth factors, the extract supports a balanced inflammatory response, facilitating the transition from the inflammatory phase to the proliferative and remodeling phases of wound healing. This multifaceted mode of action underscores the therapeutic potential of *Moringa oleifera* as a natural wound-healing agent and supports its development as an alternative or complementary therapy for wound management, particularly in conditions associated with delayed healing (Al-Ghanayem et al., 2022).

Oxidative stress and inflammatory responses play critical roles in the regulation of wound healing. Excessive production of reactive oxygen species (ROS) at the wound site can impair cellular function and delay tissue repair, while a prolonged inflammatory response may hinder the progression of healing from the inflammatory to the proliferative phase. Antioxidant enzymes such as SOD and CAT are essential in maintaining redox balance by neutralizing ROS, thereby protecting cells and supporting tissue regeneration (Ukaegbu et al., 2025).

TGF-β is a key cytokine involved in inflammation, fibroblast activation, and extracellular matrix deposition during wound healing. Although TGF-β is necessary in the early stages of repair, excessive or prolonged expression may contribute to delayed healing or abnormal tissue remodeling. Therefore, the regulation of SOD, CAT, and TGF-β expression is crucial for achieving balanced and effective wound healing (Finnson et al., 2013).

In this study, gene expression analysis was conducted to evaluate the effects of *Moringa oleifera* extract treatment on the expression levels of SOD, CAT, and TGF-β in treated wounds. This analysis provides molecular insight into the mechanisms underlying the observed wound-healing effects and supports the role of *Moringa oleifera* as a modulator of

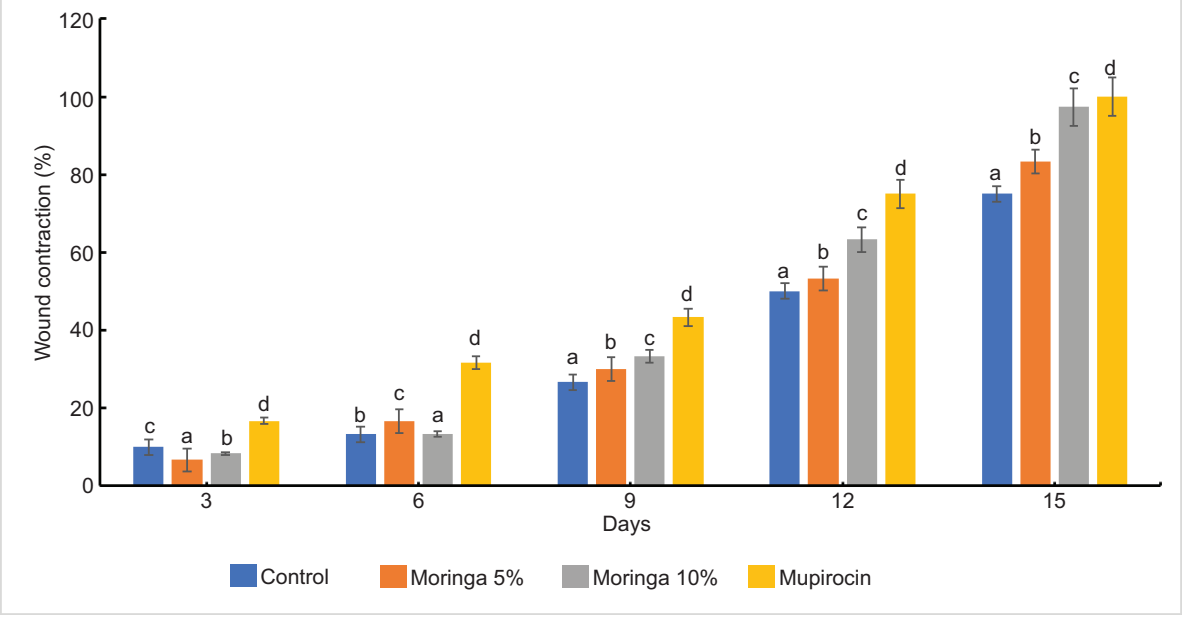

Figure 5. Percentage of wound contraction in rats treated with root extract ointment of Moringa oleifera compared to control groups over 15 days.

Note: Data were analyzed using one-way ANOVA with a significance level of $p < 0.05$. Post hoc analysis was performed using Tukey's test. Different superscript letters (a, b, etc.) indicate statistically significant differences between groups ($p < 0.05$).

oxidative stress and inflammatory responses during tissue repair (Finnson et al., 2013).

Figure 6 shows the relative mRNA expression levels of the antioxidant genes SOD and CAT in wound tissue following topical treatment with Moringa oleifera root extract. Both treatment groups exhibited significantly higher expression levels than the untreated control, indicating enhanced antioxidant defense mechanisms during the wound-healing process.

Quantitative PCR analysis conducted on day 15 revealed an upregulation of key antioxidant genes, including SOD and CAT, in wound tissues treated with *Moringa oleifera* extract compared to the control group (Figure 6). An increase in the expression of these genes indicates enhanced antioxidant defense mechanisms at the wound site, which play a critical role in mitigating oxidative stress during the healing process.

Oxidative stress is a major factor that can delay wound healing by inducing cellular damage and impairing tissue regeneration. The elevated expression of SOD and CAT suggests that *Moringa oleifera* extract effectively enhances the enzymatic neutralization of ROS, thereby protecting cells from oxidative damage and creating a more favorable microenvironment for tissue repair. This molecular response is consistent with the presence of antioxidant compounds identified in the GC–MS analysis, such as α-tocopherol, ascorbyl palmitate, and phenolic derivatives, which are known to stimulate antioxidant pathways (Gliozheni et al., 2025).

Furthermore, the modulation of healing-related genes, including TGF-β, plays an important role in coordinating inflammation, fibroblast activity, and extracellular matrix remodeling during wound repair (Qiu et al., 2025). The balanced regulation of TGF-β expression observed in the *Moringa oleifera*–treated groups suggests an optimized healing response, preventing excessive inflammation while supporting tissue regeneration.

Figure 7 illustrates the relative mRNA expression of the TGF-β1 gene in wound tissue following topical treatment with Moringa oleifera root extract. Compared with the untreated control group, both extract-treated groups showed lower TGF-β1 expression, indicating that the extract effectively modulated inflammatory and tissue-remodeling responses during wound healing. The 10% extract exhibited a stronger regulatory effect than the 5% extract, approaching the expression pattern observed in normal tissue.

As shown in Figure 7, the relative mRNA expression of the TGF-β1 gene was markedly higher in the wound tissue of the control group compared to the normal group, indicating an exaggerated inflammatory response in untreated wounds. Topical treatment with *Moringa oleifera* extract resulted in a modulation of TGF-β1 expression, with both 5% and 10% extract concentrations showing reduced expression levels compared to the control group, while remaining higher than normal tissue. This pattern suggests a regulatory effect of *Moringa*

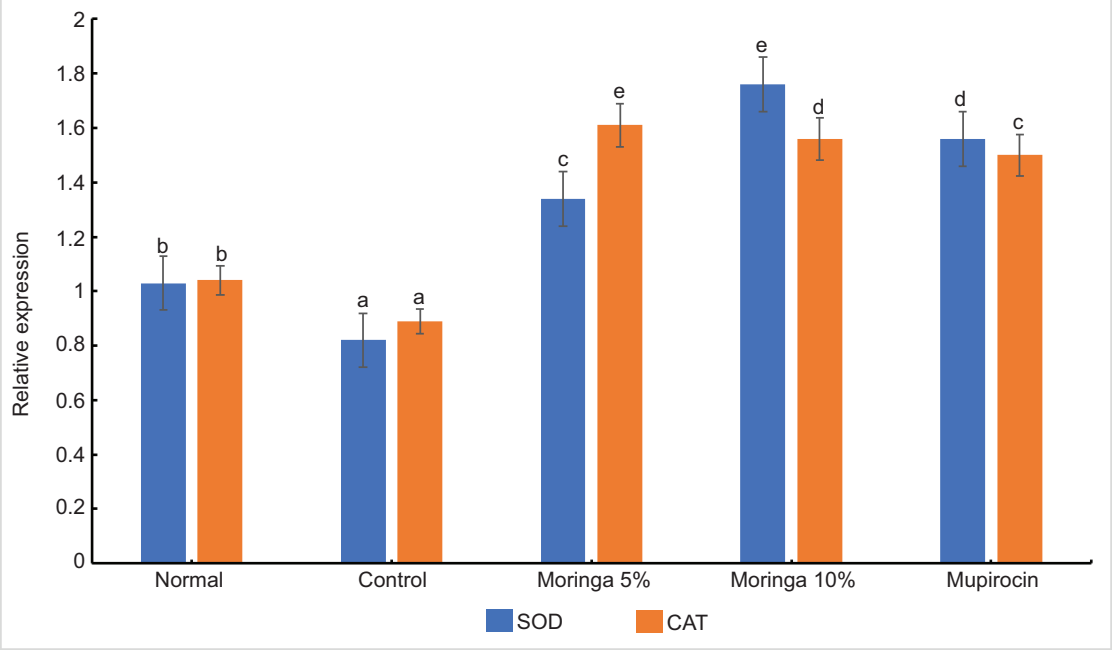

Figure 6. Relative mRNA expression levels of SOD and CAT genes in wound tissue treated with Moringa oleifera root extract.
Note: Data were analyzed using one-way ANOVA with a significance level of $p < 0.05$. Post hoc analysis was performed using Tukey's test. Different superscript letters (a, b, etc.) indicate statistically significant differences between groups ($p < 0.05$).

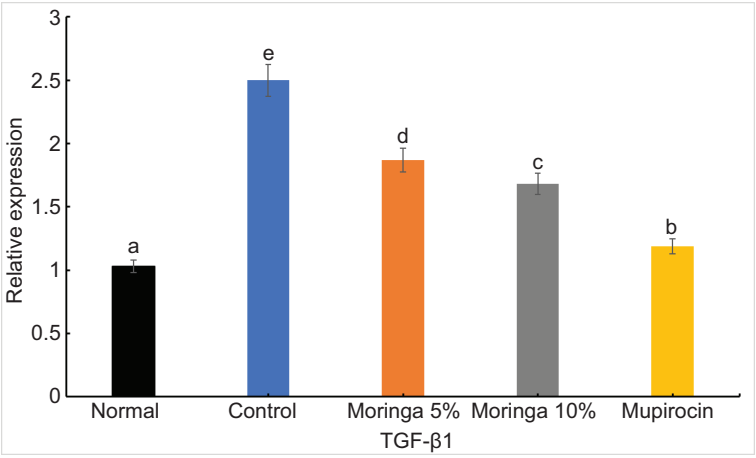

Figure 7. Relative mRNA expression of TGF-β1 gene in wound tissue after topical treatment with Moringa oleifera root extract ointment.

Note: Data were analyzed using one-way ANOVA with a significance level of $p < 0.05$. Post hoc analysis was performed using Tukey's test. Different superscript letters (a, b, etc.) indicate statistically significant differences between groups ($p < 0.05$).

oleifera on TGF-β1 expression, promoting a balanced healing response (Gliozheni et al., 2025; Qiu et al., 2025).

The modulation of TGF-β1 expression highlights the role of *Moringa oleifera* in regulating key molecular pathways involved in wound healing. TGF-β is a critical growth factor that governs fibroblast activation, collagen synthesis, and extracellular matrix production, all of which are essential for effective tissue repair (Gliozheni et al., 2025; Qiu et al., 2025). Controlled upregulation of TGF-β1 stimulates fibroblast proliferation and collagen deposition, thereby strengthening the wound matrix and facilitating the transition from the inflammatory phase to the proliferative and remodeling phases of healing.

Importantly, excessive or prolonged TGF-β expression can contribute to delayed healing or excessive scar formation. The moderated expression observed in the *Moringa oleifera*–treated groups suggests that the extract supports optimal tissue regeneration without inducing excessive fibrotic responses. This regulatory effect is consistent with the observed acceleration of wound contraction and improved healing outcomes in the in vivo model.

When considered together with the upregulation of antioxidant genes SOD and CAT, these findings indicate that *Moringa oleifera* extract promotes wound healing through a comprehensive molecular mechanism. By enhancing antioxidant defenses, the extract reduces oxidative stress at the wound site, while the modulation of TGF-β1 supports fibroblast activity and extracellular matrix remodeling. This dual action optimizes both the biochemical and cellular processes required for effective tissue repair (Gliozheni et al., 2025; Qiu et al., 2025).

Overall, the coordinated regulation of SOD, CAT, and TGF-β1 expression underscores the multifaceted wound-healing potential of *Moringa oleifera*. These molecular effects, combined with its antimicrobial properties and bioactive compounds identified through GC–MS analysis, support the potential application of *Moringa oleifera* as a natural therapeutic agent for wound management, particularly in conditions associated with impaired or delayed healing.

4. CONCLUSION

Moringa oleifera demonstrates significant effectiveness in wound healing due to its antibacterial and antioxidant properties. Active compounds such as palmitoleic acid, β-sitosterol, and oleanolic acid contribute to reducing inflammation and enhancing tissue regeneration. Integrating Moringa extracts into treatment protocols can provide a natural solution to combat infections and expedite the healing process, particularly in the context of rising antibiotic resistance. Further research is needed to isolate bioactive compounds and explore their mechanisms of action.

AUTHOR CONTRIBUTIONS

Marhaen Hardjo did research concept and design, critical revision of the article, and the final approval of the article; Surahmat Hamid did collection and/or assembly of data, data analysis and interpretation, and writing of the article; Syahrijuita Kadir performed critical revision of the article

and the final approval of the article; Ika Yustisia, Shintya Dwipuspa Rani, and Dzulrizka Razak were responsible for collection and/or assembly of data, and data analysis and interpretation; and Moh. Aqil Hardjo did collection and/or assembly of data.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' DECLARATION

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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