

ORIGINAL RESEARCH ARTICLE

Wound-healing efficacy of *Libidibia ferrea* fruit-derived topical formulations in Wistar rats

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Citation: Cerdeira AKLA, da Silva Pena FP, Ferreira CRS, *et al.* Wound-healing efficacy of *Libidibia ferrea* fruit-derived topical formulations in Wistar rats. *Global Transl Med.* 2026;5(1):30-43. doi: 10.36922/GTM025350067

Received: August 30, 2025

Revised: December 14, 2025

Accepted: December 15, 2025

Published online: January 12, 2026

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Abstract

Libidibia ferrea var. *ferrea* (Mart. ex Tul.) L.P. Queiroz (syn. *Caesalpinia ferrea*), popularly known as jucá, is widely distributed in the Amazon region and northeastern Brazil and is conventionally used to treat various diseases, including diabetes, infections, and inflammatory conditions. This study evaluated the healing effects of topical formulations derived from *L. ferrea* fruits on cutaneous wounds induced in Wistar rats. Three topical preparations were assessed: A glycolic extract of *L. ferrea*, a fruit infusion formulation (InLf), and a carbopol gel formulation (GLf). The phytochemical compounds present in the formulations, such as tannins and flavonoids, are known for their antioxidant, anti-inflammatory, and antimicrobial properties, which are essential for the healing process. Histopathological methods were used to evaluate healing, including analysis of epithelialization, granulation tissue formation, and collagen deposition. The results demonstrated that InLf and GLf significantly accelerated healing, with greater type I collagen deposition in the groups treated with these formulations, suggesting a more advanced tissue repair process. Among the formulations, InLf demonstrated the most pronounced healing effects, possibly due to its higher flavonoid content. The *in silico* study showed that the main markers of this species—gallic acid and ethyl gallate—are involved in the observed pharmacological response, modulating vascular proliferation and fibroblast activity. Overall, these findings reinforce the therapeutic potential of *L. ferrea* in the development of natural healing agents.

Keywords: Wound healing; *Libidibia ferrea*; Tannins; Flavonoids; Collagen; Skin wounds; Wistar rats

1. Introduction

Wound healing is a dynamic and complex biological process that restores tissue integrity after injury. This process involves several phases: inflammation, cell proliferation, granulation tissue formation, and remodeling.^{1,2} The development of new healing agents, particularly those derived from natural sources, has recently intensified, with a focus on medicinal plants that have a long tradition of widespread use.^{3,4}

Libidibia ferrea var. *ferrea* (Mart. ex Tul.) LP Queiroz (synonym of *Caesalpinia ferrea*), popularly known as jucá, is a plant widely distributed in the Amazon region and northeastern Brazil, and is traditionally used to treat various diseases, including diabetes, infections, and inflammatory conditions.^{5,6} Scientific studies have demonstrated that the fruits, bark, and leaves of this plant possess a wide range of pharmacological properties, including anti-inflammatory, antimicrobial, and antioxidant activities, which are essential for healing.^{7,8}

Several bioactive compounds have been isolated from *L. ferrea*, including gallic acid and methyl gallate, which have demonstrated antitumor potential in *in vitro* and *in vivo* assays, inhibiting the early activation of Epstein–Barr virus antigens and other models of carcinogenesis.^{9,10} Furthermore, the aqueous extract of *L. ferrea* has demonstrated beneficial effects, including protection against gastric ulcers in experimental models, reduced gastric secretion volume, and promotion of tissue regeneration.^{11,12}

The wound-healing activity of medicinal plants is of particular interest for the development of topical pharmaceutical formulations, such as gels and creams, that can accelerate wound healing and reduce the risk of complications, including infections.^{3,13} In previous studies, the crude extract of *L. ferrea* demonstrated potent anti-inflammatory activity in carrageenan-induced edema models and analgesic properties, suggesting its use in managing pain associated with the inflammatory process.⁷ In addition to its antimicrobial activity against oral pathogens, such as *Candida albicans* and *Streptococcus mutans*, these effects indicate that *L. ferrea* may effectively prevent infections during healing.¹⁴ A literature review on the *L. ferrea* highlighted the need for studies investigating its healing activity.¹⁵

Furthermore, *L. ferrea* has demonstrated positive effects on immune regulation, particularly by stimulating myelopoiesis in infection and tumor models, which may enhance the immune response during wound healing.¹⁶ This is particularly relevant for the clinical use of the plant, as an adequate immune response is crucial for tissue

regeneration and the prevention of complications during the healing process.¹

The present study aims to evaluate the wound-healing effects of two topical preparations of *L. ferrea* fruits, namely an Infusion formulation (InLf) and a Gel formulation (GLf) containing glycolic extract of *L. ferrea* (GcLf), in cutaneous wounds induced in Wistar rats. Previous studies indicate that the plant has great potential for wound healing due to its anti-inflammatory, antimicrobial, and immunomodulatory properties.^{7,8,15} The evaluation of these pharmaceutical formulations aims to explore the therapeutic potential of *L. ferrea* for wound treatment and to contribute to the development of new natural healing agents.

2. Materials and methods

2.1. Plant material

The fruits of *L. ferrea* were collected from the Medicinal Plant Garden of the Institute of Studies and Research of the State of Amapá, from a specimen previously identified and cataloged in the Herbarium of the Agronomic Institute of the North (IAN185211, Fabaceae: *L. ferrea* var. *ferrea* [Mart. ex Tul.]) LP Queiroz (Figure 1).

The *L. ferrea* species was used to extract GcLf for the preparation of InLf and GLf. The methods employed followed those described in the Phytotherapeutic Formulary of the Brazilian Pharmacopeia.¹⁷ Tannins and flavonoids in the preparations were quantified using the method described by Gonzalez.¹⁸

2.2. Preparation of topical formulations

The GLf were prepared using a Carbopol base. GcLf of *L. ferrea* was incorporated into the base at a concentration of 5% (w/w). The choice of this concentration was based on



Figure 1. *Libidibia ferrea* collected from the Medicinal Plant Garden of the Institute of Studies and Research of the State of Amapá. The specimen was previously identified and cataloged in the Herbarium of the Agronomic Institute of the North (IAN185211, Fabaceae: *Libidibia ferrea* var. *ferrea* [Mart. ex Tul.] LP Queiroz).

previous pilot studies that demonstrated optimal stability and efficacy without causing skin irritation.¹⁵ Total tannin and flavonoid contents were determined using the Folin–Ciocalteu and aluminum chloride colorimetric methods, respectively, as described by Gonzalez.¹⁸

2.3. Animals used in the study

The Research Ethics Committee of the Federal University of Amapá approved the study under protocol no. 008/2017 of 08/23/2017. A total of 20 male Wistar rats, albino variety, weighing 300 ± 40 g, were obtained from the Laboratory Animal Science at the Multidisciplinary Center for Biological Research, University of Campinas. The rats were 21 days old and acclimated in polyethylene cages under controlled temperature conditions ($22 \pm 3^\circ\text{C}$), with free access to food and water ad libitum, under a 12-h light/12-h dark cycle. After the acclimation period, the animals were individually placed in metabolic cages.

Each experimental group consisted of five animals, housed in polyethylene cages in an air-conditioned room at $25 \pm 2^\circ\text{C}$. The animals were deprived of food for 24 h before the experiment, but had access to water ad libitum. They received standardized food (Labina®) and water throughout the experiment, with a 12-h light/dark cycle maintained for 7 days.

2.4. Experimental groups for healing activity

Animals were allocated to four groups ($n = 5$ per group), balanced for body weight, as follows:

- (i) The first group was treated with GLf at a concentration of 5% topically, with a dose of 188 mg/kg.
- (ii) The second group (positive control) was treated topically with fibrinolysin (Fbn; Cristália Chemical Pharmaceutical Products Ltd., Brazil), with a dose of 31 mg/kg.
- (iii) The third group was treated with InLf at a concentration of 5%, at a dose of 182 mg/kg.
- (iv) The fourth group, defined as the negative control (Con), was treated with 0.5 mL of a 0.9% saline solution (sodium chloride).

Different treatments were applied to the wounds immediately after the surgical procedure and administered daily, topically, using a disposable spatula, at the same time each day.

2.5. Surgical procedure and morphometric evaluation

All surgical procedures were performed under general anesthesia, using 45 mg/kg of sodium thiopental (Cristália, Brazil) administered intraperitoneally, as described by Alves Barros *et al.*¹⁹

After shaving the dorsocervical region, a rectangular area of approximately $3\text{ cm} \times 5\text{ cm}$ was outlined using an electric clipper. The skin was disinfected with iodized alcohol. A 6-mm full-thickness excisional wound was created on the cervical region of the animal's back using a skin biopsy punch. The tissue was removed with scissors and forceps until the dorsal muscle fascia was exposed. Immediately after hemostasis, treatments began, administered once daily at standardized times (12:00–13:00) for 7 days, with the wounds remaining without dressings throughout the experiment.

The wounds were measured in a standardized manner in the craniocaudal direction, from the 1st day (immediately after surgery) to the seventh postoperative day, using a digital caliper. The rate of wound healing was analyzed. Based on the measured values, wound contraction was calculated using the following formula:

$$\text{Wound contraction percentage (\%)} = \frac{\text{Initial area} - \text{final area}}{\text{Initial area}} \times 100 \quad (1)$$

Digital images of the wounds were obtained in a standardized manner immediately after the surgical procedure (day 1) and continued until the seventh postoperative day. For this purpose, a camera (Sonic 7.0, Brazil) was used, attached to a support fixed perpendicular to the horizontal plane and kept at a distance of 8 cm from this plane, providing adequate wound focusing. The digitized images were stored in Windows Bitmap (BMP) format and analyzed in ImageLab Software Version 6.1 (Bio-Rad Laboratories Inc., Brazil).

On the 7th day after the surgical procedure, the animals were anesthetized, placed in a carbon dioxide chamber, and euthanized. Following sacrifice, the wound was excised with a 0.5-mm margin of intact skin around the lesion, extending down to the muscle fascia, under aseptic conditions, making a median cut, and the tissue was fixed in 10% formalin.

2.6. Histopathological evaluation of wounds

The histopathological evaluation was performed according to the method described by Alves Barros *et al.*¹⁹ The skin fragments fixed in 10% formalin were dehydrated and embedded in paraffin. They were then cut at $5\text{ }\mu\text{m}$ and stained with hematoxylin-eosin (HE) and picrosirius. The quantitative and qualitative analyses of the effects of both GLf and InLf included counting fibroblasts and inflammatory cells and assessing angiogenesis (blood vessels) on histopathological slides at $\times 400$ magnification.

To determine epithelialization rates (ER), histological sections stained with HE were used to evaluate the extent

of epithelialization in wounds by measuring epidermal migration from the average wound margin to the point where progression stopped. ER was calculated as the percentage of wound length covered by newly formed epithelium, determined in four high-power fields ($\times 400$) on each slide using morphometry software (Imagetool®, ImageTool 3.0 - Informer Technologies, Inc., USA), according to the equation as follows:

$$ER(\%) = \left(1 - \frac{W_A - E_A}{E_A}\right) \times 100 \quad \left\{ \begin{array}{l} ER(\%) - \text{Epithelialization rates} \\ W_A - \text{Wound surface area} \\ E_A - \text{Epithelialized area} \end{array} \right. \quad (2)$$

For the assessment of collagen deposition, histological sections stained with picrosirius and analyzed under polarized light were used for descriptive evaluation. Collagen fibers were analyzed according to their birefringence pattern (greenish/yellow-greenish or orange, orange reddish), morphological appearance (wavy or stretched, thin or thick, short or long), and disposition (parallel-arranged or interlaced).

2.7. Statistical analysis

All experimental quantitative results were expressed as mean $\pm$ standard deviation, and one-way analysis of variance was used, followed by Tukey's test for multiple comparisons. Results with significance levels of $p < 0.05$ were considered statistically significant. Statistical analyses were conducted using GraphPad Prism (version 5.03, GraphPad Software, Inc., San Diego, California, USA).

2.8. Molecular docking

The three-dimensional (3D) structure of the vascular endothelial growth factor receptor (VEGFR)-2/vascular endothelial growth factor (VEGF)-A complex (PDB ID: 3V2A) was retrieved from the Protein Data Bank (<https://www.rcsb.org/>). Initially, water molecules and cofactors were excluded, and hydrogen atoms were added using AutoDockTools 4.0 (Molecular Graphics Lab, The Scripps Research Institute, La Jolla, California, USA). The 3D models of natural product compounds were downloaded in SDF format from PubChem and converted to PDB format using Discovery Studio Visualizer (Dassault Systèmes BIOVIA, Massachusetts, USA) for subsequent docking simulations.

Before performing docking, the grid center for each protein target was determined using the AutoGrid program in AutoDockTools. The grid size was set to 40 points per dimension, with a spacing of 0.375 Å across all simulations. Molecular docking was conducted using AutoDock Vina 1.2.5 (The Scripps Research Institute, La

Jolla, California, USA)²⁰ to calculate the binding affinities of each ligand to the target protein. The number of output poses (modes) was set to 10, and the energy range was adjusted to 9 kcal/mol. To improve docking accuracy, the exhaustiveness parameter was set to 1,000. Two-dimensional and 3D visuals were generated using BIOVIA Discovery Studio Visualizer software (Dassault Systèmes BIOVIA, Massachusetts, USA).²¹

2.9. Density functional theory computational analysis

Density functional theory calculations were performed using the B3LYP functional with the 6-311G(d,p) basis set. This approach was employed to evaluate the properties of gallic acid and ethyl gallate. The B3LYP method is widely recognized for its cost-effectiveness and reliability in predicting the geometries of both natural and synthetic molecules in quantum chemistry. All computations were conducted using the GAUSSIAN 09W software package (Gaussian, Inc., Wallingford, Connecticut, USA), and the optimized molecular structures were visualized with GaussView 6.0 (Semichem Inc., Kansas, USA).²²

3. Results

3.1. Phytochemical assay and evaluation of wound contraction

Phytochemical analyses (Table 1) indicated that the GcLf of the fruits of *L. ferrea* presented a tannin content of 23% and flavonoids of 0.25%, while InLf had 21% tannins and 0.32% flavonoids. GLf demonstrated the lowest contents, with 18% tannins and 0.14% flavonoids.

The results of topical treatments on wound healing (Figure 2) showed that both InLf and GLf from *L. ferrea* significantly reduced the wound area at all measured time points ($p < 0.001$), indicating that the treatments accelerated tissue repair. The area under the curve data showed that the treatments differed significantly from the control group (Con). The difference was observed mainly in the later days of treatment, where the difference from the control group was more pronounced.

Table 1. Tannin and flavonoid content in glycolic extract, infusion, and gel of *Libidibia ferrea*

Product	Tannin (%)	Flavonoid (%)
GcLf	23	0.25
InLf	21	0.32
GLf	18	0.14

Abbreviations: GcLf: Glycolic extract of *Libidibia ferrea*; GLf: Gel formulation; InLf: Infusion formulation.

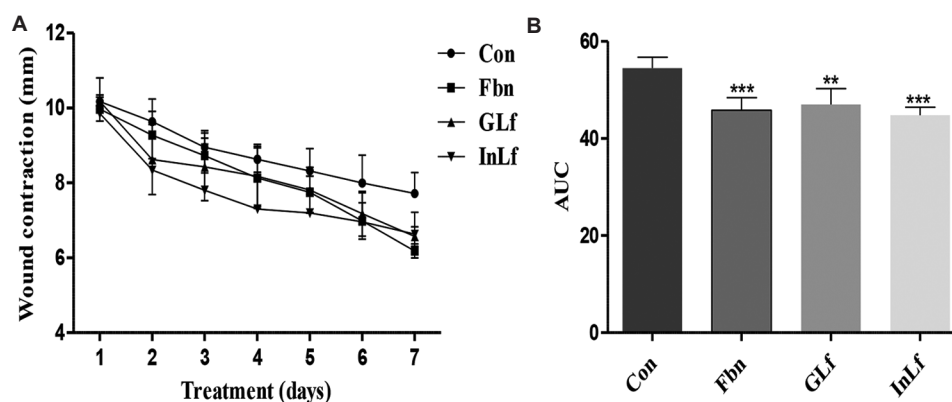


Figure 2. Effect of topical administration of GLf (188 mg/kg), InLf (182 mg/kg), and Fbn (31 mg/kg) on cutaneous wound healing. (A) Wound contraction throughout treatment. (B) AUC for each group.

Notes: Data are presented as mean±standard deviation. ** $p<0.01$ and *** $p<0.001$ versus Con group, according to Tukey's *post hoc* test following one-way analysis of variance.

Abbreviations: AUC: Area under the curve; Con: Control; Fbn: Fibrinolysin; GLf: Glycolic extract of *Libidibia ferrea*; GLf: Gel formulation; InLf: Infusion.

3.2. Histopathological evaluation of wounds

The findings revealed that the GLf group (Figure 3) showed a moderate inflammatory infiltrate in the marginal portion of the wound, predominantly lymphocytic, as well as intense proliferation of plump fibroblasts organized in parallel, associated with the deposition of rectilinear, delicate bundles of collagen exhibiting varying thickness. The vascular component was represented by capillary vessels, some of which were congested. The surface of the wound was covered with acanthotic orthokeratinized squamous epithelium. The most central portion of the wound exhibited a superficial third of the wound bed, representing a prominent, highly vascular granulation reaction. The deepest third of the wound bed exhibits a mature fibrocellular granulation reaction, characterized by moderate lymphocytic infiltration, fibroblastic proliferation, and collagen deposition, similar to the pattern observed at the margins.

In the marginal portion, Figure 4 shows that the Fbn group exhibited moderate inflammatory infiltration and a granulation reaction, with a less significant vascular-capillary component. The connective tissue appeared denser, consisting of thin, short fibrils, accompanied by intense fibroblast proliferation. In addition, clear epithelialization at the wound edges was observed. In the central region, a fibrovascular granulation reaction was observed. The connective tissue consisted of a highly loose extracellular matrix composed of thin, delicate, and very short collagen fibrils. Peripheral epithelialization was also visible.

Figure 5 shows that the InLf group exhibited a moderate lymphohistiocytic inflammatory infiltrate, along with intense proliferation of plump fibroblasts arranged parallel

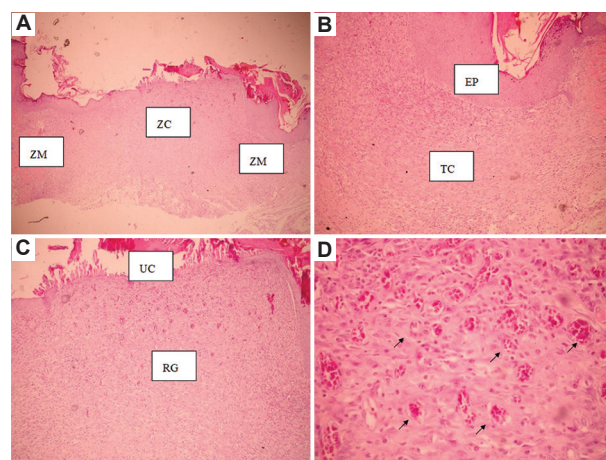


Figure 3. Histopathological evaluation of wounds treated with the gel formulation (188 mg/kg). (A) Overall view of the wound, highlighting the ZM and ZC zones (scale bar: 250 μ m; magnification: $\times 40$). (B) ZM zone, highlighting an area of EP and underlying inflamed TC (scale bar: 100 μ m; magnification: $\times 100$). (C) ZC zone, highlighting the RG and UC zone without epithelial lining (scale bar: 50 μ m; magnification: $\times 100$). (D) Vascular components (denoted with arrows) in the superficial region (scale bar: 25 μ m; magnification: $\times 400$).

Abbreviations: EP: Epithelialization; RG: Granulation reaction; TC: Connective tissue; UC: Ulcerated zone; ZC: Central zone; ZM: Marginal zone.

to the wound surface. These features are associated with the deposition of straight and delicate collagen bundles of varying thickness. Well-defined marginal epithelialization was present, accompanied by evident acanthosis of the newly formed epithelium. In the central portion of the wound, an immature, predominantly fibrovascular granulation reaction extended from the surface toward the deeper layers.

Figure 6 shows that, in the Con group, the marginal portion exhibited a lymphohistiocytic inflammatory

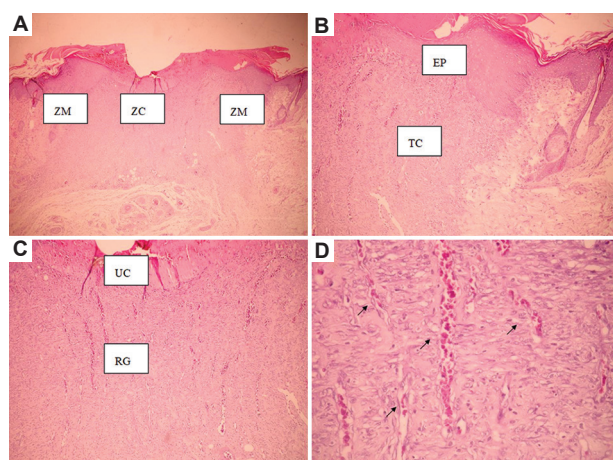


Figure 4. Histopathological evaluation of wounds treated with fibrinolysin (31 mg/kg). (A) Overall view of the wound, highlighting the ZM and ZC zones (scale bar: 250 µm; magnification: ×40). (B) ZM zone, highlighting an area of EP and underlying inflamed TC (scale bar: 100 µm; magnification: ×100). (C) ZC zone, highlighting the RG and UC zone without epithelial lining (scale bar: 50 µm; magnification: ×100). (D) Vascular components (denoted with arrows) in the superficial region (scale bar: 25 µm; magnification: 400×).

Abbreviations: EP: Epithelialization; RG: Granulation reaction; TC: Connective tissue; UC: Ulcerated zone; ZC: Central zone; ZM: Marginal zone.

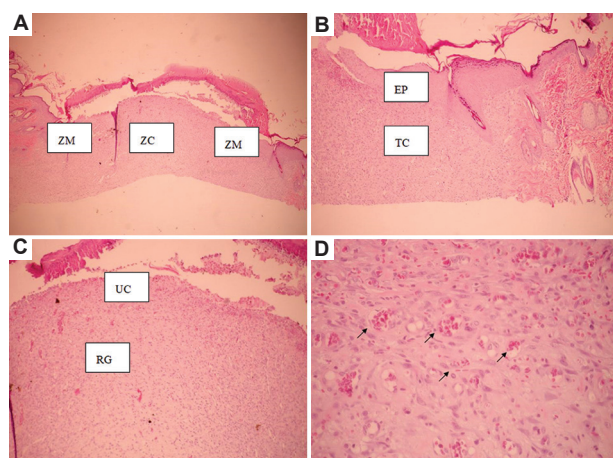


Figure 5. Histopathological evaluation of wounds treated with the infusion formulation (182 mg/kg). (A) Overall view of the wound, highlighting the ZM and ZC zones (scale bar: 250 µm; magnification: ×40). (B) ZM zone, highlighting an area of EP and underlying inflamed TC (scale bar: 100 µm; magnification: ×100). (C) ZC zone, highlighting the RG and UC zone without epithelial lining (scale bar: 50 µm; magnification: ×100). (D) Vascular components (denoted with arrows) in the superficial region (scale bar: 25 µm; magnification: ×400).

Abbreviations: EP: Epithelialization; RG: Granulation reaction; TC: Connective tissue; UC: Ulcerated zone; ZC: Central zone; ZM: Marginal zone.

reaction with a prominent vascular component of the granulation tissue. However, the vasculature consisted of small, poorly formed capillaries with irregular morphology

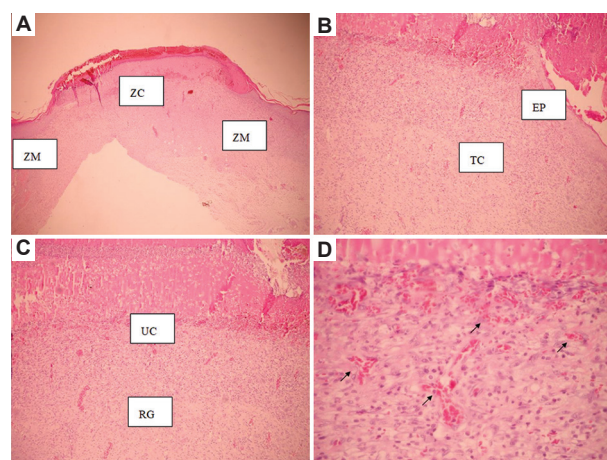


Figure 6. Histopathological evaluation of untreated (control) wounds. (A) Overall view of the wound, highlighting the ZM and ZC zones (scale bar: 250 µm; magnification: ×40). (B) ZM zone, highlighting an area of EP and underlying inflamed TC (scale bar: 100 µm; magnification: ×100). (C) ZC zone, highlighting the RG and UC zone without epithelial lining (scale bar: 50 µm; magnification: ×100). (D) Vascular components (denoted with arrows) in the superficial region (scale bar: 25 µm; magnification: ×400).

Abbreviations: EP: Epithelialization; RG: Granulation reaction; TC: Connective tissue; UC: Ulcerated zone; ZC: Central zone; ZM: Marginal zone.

(“slit-like” vessels), accompanied by moderate fibroblast proliferation and deposition of short, delicate fibrillar collagen, arranged in widely spaced parallel distribution, typical of highly immature connective tissue.

In the central portion, a richly vascularized granulation reaction extended from the surface to the deeper layers, along with moderate interstitial edema. Persistent superficial neutrophilic infiltration and moderate interstitial edema were observed in all specimens.

3.3. Assessment of epithelialization, angiogenesis, and inflammatory cells

The ER (Figure 7A) in the GLf- and Fbn-treated groups was significantly higher ($p < 0.001$) than in the Con group. Moreover, the InLf-treated group also showed a significant difference ($p < 0.05$) compared to the Con group.

In the microscopic analysis at ×400 magnification, the presence of inflammatory cells (Figure 7B) showed that the mean cell count in the Con group was significantly higher than in the other groups. In the groups treated with GLf, InLf, and Fbn, significantly lower values were observed ($p < 0.001$), with means of 9.1 ± 4.48 , 11 ± 3.22 , and 11.2 ± 3.28 , respectively.

Regarding angiogenesis (Figure 7C), a highly significant difference was observed in the mean values of the three groups compared to the Con group. For example, in the GLf-treated group, a significant increase ($p < 0.001$) in

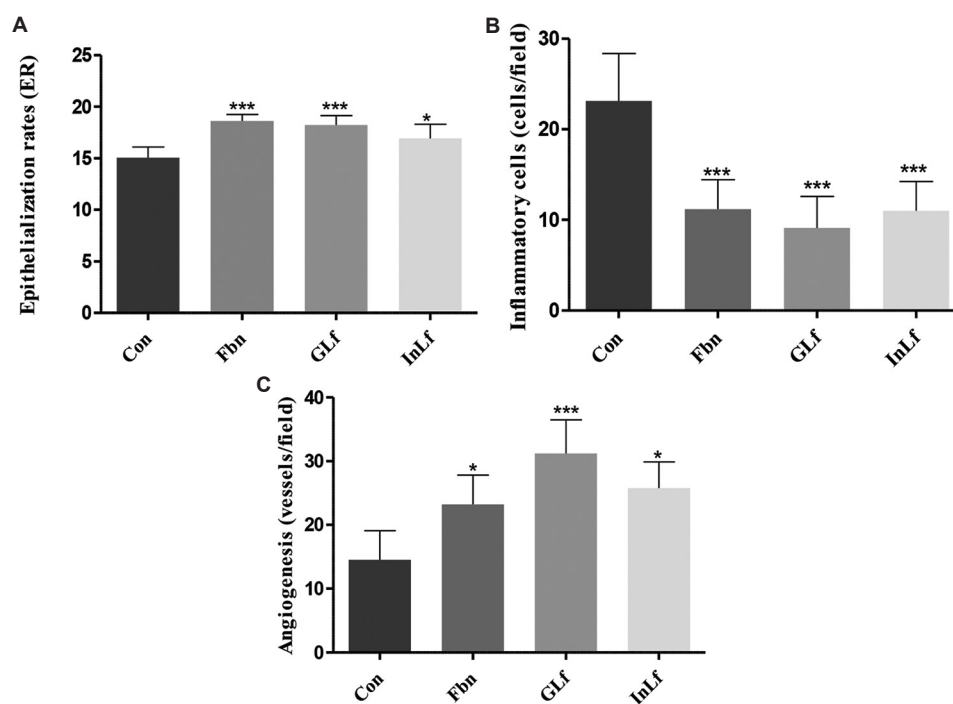


Figure 7. Results of histological assessments. (A) Epithelialization rate of the GLf (188 mg/kg), InLf (182 mg/kg), and Fbn (31 mg/kg) groups. (B) Number of inflammatory cells (cells/field). (C) Angiogenesis values (vessels/field) of the treated groups.

Notes: * $p < 0.05$ and *** $p < 0.001$ versus Con group, according to Tukey's *post hoc* test following one-way analysis of variance.

Abbreviations: Con: Control; Fbn: Fibrinolysin; GLf: Glycolic extract of *Libidibia ferrea*; GLf: Gel formulation; InLf: Infusion.

the mean number of vessels per field was observed (31.2 ± 5.27). In groups treated with Fbn and InLf, significant differences ($p < 0.05$) were observed when compared to the Con group, with mean values of 23.2 ± 4.62 and 25.8 ± 4.08 , respectively.

3.4. Quantification of fibroblasts and collagen deposition

Fibroblast quantification in histopathological slides (Figure 8) revealed a significant increase ($p < 0.01$) was observed in the average number of new fibroblasts in the GLf-treated group, with a value of 27.88 ± 5.24 cells/field. In the groups treated with InLf and Fbn, significant differences ($p < 0.05$) were also observed compared to the Con group. The Con group exhibited significantly reduced fibroblast proliferation, with an average value of 13.2 ± 4.6 cells/field.

The analysis of collagen deposition in histopathological sections stained with picrosirius and examined under polarized light microscopy (Figure 9) revealed that, in the GLf and Fbn groups, short, thin, and delicate fibrous bundles with a slightly wavy appearance, irregular arrangement, and greenish to yellow-green birefringence were observed, consistent with deposition of reticular type III collagen. The bundles were more abundant in deeper regions and at the

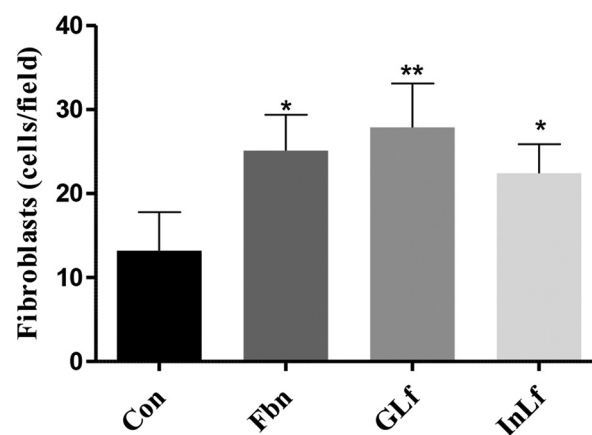


Figure 8. Fibroblast proliferation (cells/field) in groups treated with GLf (188 mg/kg), InLf (182 mg/kg), and Fbn (31 mg/kg).

Notes: * $p < 0.05$ and ** $p < 0.01$ versus Con group, according to Tukey's *post hoc* test following one-way analysis of variance

Abbreviations: Con: Control; Fbn: Fibrinolysin; GLf: Glycolic extract of *Libidibia ferrea*; GLf: Gel formulation; InLf: Infusion.

wound edges, and fibrils with yellow-orange birefringence, compatible with type I collagen, were also observed. Interfibrillar spaces were plentiful. The pattern observed in the InLf group was similar, but type I collagen deposition was minimal; even in the marginal zones, this collagen was

scarce. In contrast, in the Con group, only type III collagen was observed along the entire wound, with the bundles remaining relatively thin and arranged in a reticular pattern.

3.5. Molecular docking

The binding interactions of gallic acid and ethyl gallate with the target protein 3V2A were analyzed, revealing significant differences in their binding energy, ligand

efficiency, and estimated inhibition constants (Table 2). Gallic acid exhibited a binding energy of -5.5 kcal/mol, ligand efficiency of -0.460 , and an inhibition constant (K_i) of 92.7 μM . In contrast, ethyl gallate demonstrated slightly higher binding affinity, with a binding energy of -5.6 kcal/mol, a ligand efficiency of -0.400 , and a K_i of 83.9 μM . These results indicate that ethyl gallate may form more stable interactions with the target protein than gallic acid. However, the differences in ligand efficiency suggest that gallic acid achieves more effective binding per heavy atom, despite ethyl gallate having a lower K_i value, indicating stronger overall inhibitory potential. This finding highlights the significance of functional modifications in shaping molecular interactions with protein targets.

Docking analysis of gallic acid and ethyl gallate with the 3V2A protein revealed distinct interaction profiles reflecting their binding characteristics (Table 3). Gallic acid formed several conventional hydrogen bonds, notably with R: LYS286 (2.16 Å) and A: PHE47 (1.91 Å, 2.22 Å), along with an interaction with R: ASN253 (2.69 Å). It also exhibited carbon-hydrogen bonds with R: LYS286 (2.78 Å), and π -donor hydrogen bonding and π -sigma interactions with A: PHE47 (3.27 Å) and A: ILE46 (2.06 Å), respectively (Figure 10).

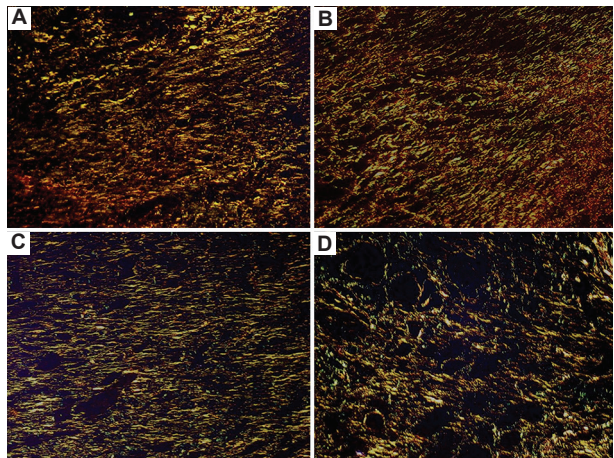


Figure 9. Collagen deposition under polarized light microscopy (scale bar: 100 μm ; magnification: $\times 100$). Groups treated with (A) GLf (188 mg/kg) and (B) Fbn (31 mg/kg) exhibited a predominance of type III collagen fibers (greenish-yellow birefringence), with evident deposition of type I collagen fibers (orange birefringence) in the deeper lamina, arranged predominantly in parallel bundles. (C) The InLf-treated group (182 mg/kg) and (D) the Con group exhibited exclusively type III collagen fibers, showing a parallel arrangement in the InLf group and a reticular pattern in the Con group. Abbreviations: Con: Control; Fbn: Fibrinolysin; GcLf: Glycolic extract of *Libidibia ferrea*; GLf: Gel formulation; InLf: Infusion.

Table 2. Results of binding interactions of the compounds with the target protein (3V2A)

Bioactive compound	Binding energy (kcal/mol)	Ligand efficiency	Estimated inhibition constant (K_i μM)
Gallic acid	-5.5	-0.460	92.7
Ethyl gallate	-5.6	-0.400	83.9

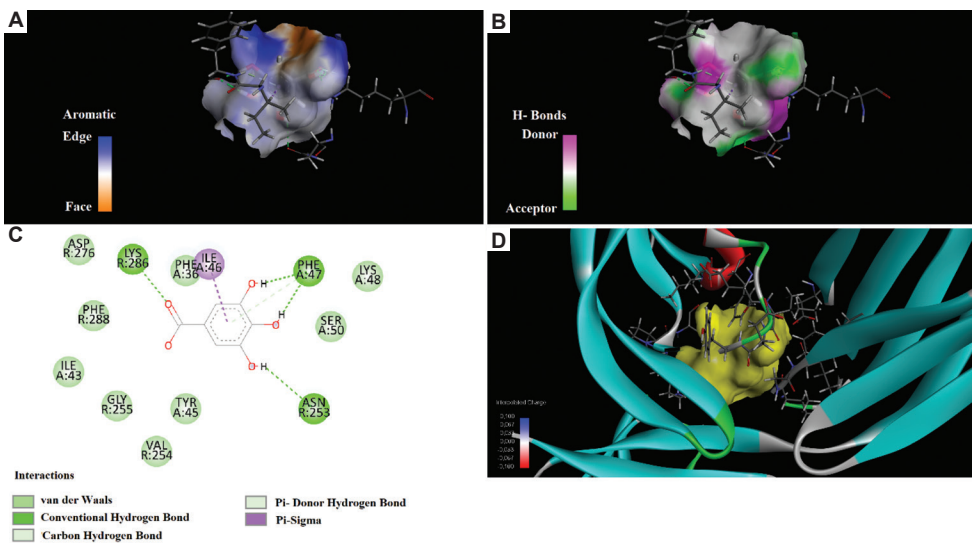


Figure 10. Molecular interactions of gallic acid with the target protein 3V2A: (A) Aromatic surface representation, (B) hydrogen bond distribution, (C) two-dimensional ligand-protein interaction, and (D) three-dimensional representation of the docked poses

Table 3. Predicted interactions of docked conformations of gallic acid and ethyl gallate with the 3V2A structure

Ligand	Amino acid	Interaction	Distance (Å)
Gallic acid	R: LYS286:HZ1 - :[001:O5	Conventional hydrogen bond	2.16
	: [001:H1 - A: PHE47:O	Conventional hydrogen bond	1.91
	: [001:H2 - A: PHE47:O	Conventional hydrogen bond	2.22
	: [001:H3 - R: ASN253:OD1	Conventional hydrogen bond	2.69
	R: LYS286:HE1 - : [001:O5	Carbon-hydrogen bond	2.78
	A: PHE47:HN - : [001	π -donor hydrogen bond	3.27
Ethyl gallate	A: ILE46:HB - : [001	π -sigma	2.06
	: [001:H7 - A: PHE47:O	Conventional hydrogen bond	1.79
	: [001:H8 - A: ASP34:OD1	Conventional hydrogen bond	1.81
	: [001:H9 - A: ASP34:OD1	Conventional hydrogen bond	2.58
	R: ASN253:HA - : [001:O2	Carbon-hydrogen bond	2.58
	: [001:H5 - A: TYR45:O	Carbon-hydrogen bond	2.93
	A: PHE36 - : [001	π - π T-shaped	4.29
	: [001:C3 - A: ILE46	Alkyl	4.67
	A: PHE36 - : [001:C3	π -alkyl	4.03

In contrast, ethyl gallate demonstrated a broader range of interactions, including hydrogen bonds with A: PHE47 (1.79 Å) and A: ASP34 (1.81 Å, 2.58 Å), as well as a carbon-hydrogen bond with R: ASN253 (2.58 Å). Furthermore, ethyl gallate displayed π - π T-shaped interactions with A: PHE36 (4.29 Å), alkyl interactions with A: ILE46 (4.67 Å), and π -alkyl bonding with A: PHE36 (4.03 Å) (Figure 11).

These findings suggest that ethyl gallate forms a more diverse set of non-covalent interactions due to its additional ethyl group, potentially contributing to its slightly enhanced binding affinity relative to gallic acid. This comparison highlights the impact of structural modifications on binding properties and potential biological activity.

3.6. Density functional theory analysis

The density functional theory analysis of gallic acid and ethyl gallate provides valuable insights into their electronic properties, which influence their reactivity and interaction potential (Table 4). Both compounds exhibited the same highest occupied molecular orbital energy (EHOMO) value of -0.2289 eV, indicating similar tendencies to donate electrons. However, their lowest unoccupied molecular orbital energy (ELUMO) values differ slightly, with gallic acid at -0.0535 eV and ethyl gallate at -0.0461 eV, suggesting that ethyl gallate has a marginally higher electron affinity. This is further supported by their $\Delta E(\text{ELUMO-EHOMO})$ values, which are 0.1754 eV for gallic acid and 0.1828 eV for ethyl gallate, indicating a

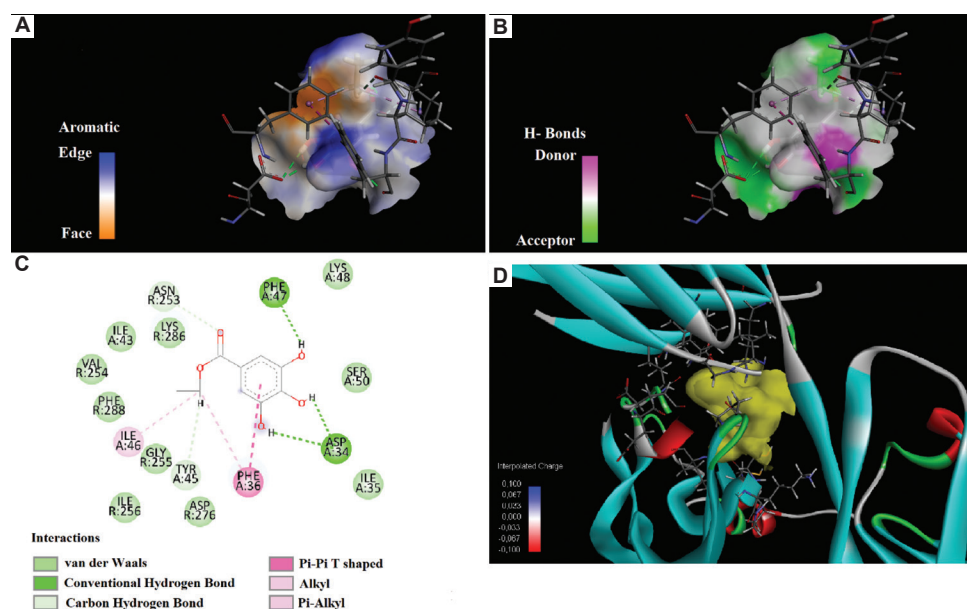


Figure 11. Molecular interactions of ethyl gallate with the target protein 3V2A: (A) Aromatic surface representation, (B) hydrogen bond distribution, (C) two-dimensional ligand-protein interaction, and (D) three-dimensional representation of the docked poses

slightly larger energy gap for ethyl gallate and potentially higher chemical stability (Figure 12).

The ionization energy (I) of both compounds is identical at 0.2289 eV, while their electron affinities (A) are 0.0535 eV and 0.0461 eV for gallic acid and ethyl gallate, respectively. The electronegativity index (χ) shows a slight difference, with gallic acid having a value of 0.1412 eV compared to 0.1375 eV for ethyl gallate. Additionally, their chemical potentials (μ) are -0.1412 eV and -0.1375 eV, respectively.

In terms of hardness (η), gallic acid exhibited a slightly lower value (0.0877 eV) compared to ethyl gallate (0.0914 eV), while their softness values, calculated as $1/\eta$, are 11.40 eV $^{-1}$ and 10.94 eV $^{-1}$, respectively. Finally, the electrophilicity index (ω), which assesses the compound's propensity to accept electrons, was marginally higher

for gallic acid (0.1137 eV) compared to ethyl gallate (0.1034 eV).

Overall, the subtle differences in electronic parameters highlight how the addition of an ethyl group to gallic acid affects its electronic properties, potentially altering its reactivity and interaction with biological targets.

4. Discussion

Wound healing is a complex process involving multiple phases, including inflammation, granulation tissue formation, and collagen remodeling. This study investigated the effects of topical application of products containing *L. ferrea* fruit derivatives on the healing process in cutaneous wounds, correlating the histopathological findings with the phytochemical composition of the formulations, specifically their tannin and flavonoid contents.

Phytochemical analyses revealed that the formulations had high tannin contents of 18–23% and flavonoid contents of 0.14–0.25%. Tannins and flavonoids are widely recognized for their antioxidant and anti-inflammatory properties, suggesting that formulations richer in these compounds may facilitate the healing process by modulating inflammation and protecting cells from oxidative stress.^{3,6}

Topical *L. ferrea* formulations significantly reduced wound size and promoted lesion contraction in rats compared to the Con group. Wound contraction is a key process contributing to wound closure. It involves re-epithelialization, epithelial cell migration, fibroplasia, and angiogenesis, with granulation tissue occupying the injured tissue approximately 4 days after the injury.^{22,23}

In the histopathological evaluation, on the 7th day, the inflammatory reaction was intense in all groups, as expected in wound healing by secondary intention, where inflammation is a necessary step for eliminating harmful agents and initiating the repair process.¹ However, distinct differences in the inflammatory pattern were observed between the groups. In the groups treated with *L. ferrea* (GLf and InLf) and Fbn, inflammation progressively decreased throughout the treatment, favoring wound closure. This suggests a rapid transition from acute to controlled inflammation and a decrease in inflammatory cells within the scar tissue, a known effect of *L. ferrea* formulations.²⁴ The high concentration of tannins in GLf and InLf modulated the inflammatory response, reduced the need for continuous neutrophil recruitment, and accelerated the transition to the proliferative phase, thereby promoting wound closure.²⁵

During the proliferative phase, granulation tissue is characterized by the presence of fibroblasts and newly

Table 4. Key parameters in density functional theory analysis and their values

Parameters	Gallic acid	Ethyl gallate
EHOMO (eV)	−0.2289	−0.2289
ELUMO (eV)	−0.0535	−0.0461
ΔE (LUMO–HOMO) (eV)	0.1754	0.1828
Ionization energy (I) = $-E$ (HOMO) (eV)	0.2289	0.2289
Electron affinity (A) = $-E$ (LUMO) (eV)	0.0535	0.0461
Electronegativity index (χ) = $(I+A)/2$ (eV)	0.1412	0.1375
Chemical potential $\mu = -\chi$	−0.1412	−0.1375
Chemical hardness $\eta = (I-A)/2$ (eV)	0.0877	0.0914
Softness $(1/\eta)$ (eV $^{-1}$)	11.40	10.94
Electrophilicity index ($\omega = \mu^2/2\eta$)	0.1137	0.1034

Abbreviations: EHOMO: Highest occupied molecular orbital energy; ELUMO: Lowest unoccupied molecular orbital energy; HOMO: Highest occupied molecular orbital; LUMO: Lowest unoccupied molecular orbital.

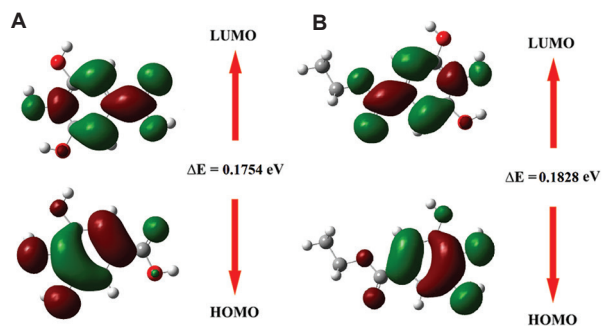


Figure 12. Frontier molecular orbital (HOMO–LUMO) distribution of (A) gallic acid and (B) ethyl gallate

Abbreviations: HOMO: Highest occupied molecular orbital; LUMO: Lowest unoccupied molecular orbital.

formed vessels. This phase was most pronounced in the groups treated with GLf, Fbn, and InLf, which presented higher rates of epithelialization and increased angiogenesis compared to the Con group. Angiogenesis is crucial for wound healing, as the newly formed blood vessels supply oxygen and nutrients necessary for tissue repair.²⁶

The epithelialization process, which involves the proliferation and migration of epithelial cells to cover the injured area, was more advanced in the GLf, Fbn, and InLf groups than in the Con group. This suggests that formulations rich in tannins and flavonoids may accelerate epithelialization, possibly by modulating the inflammatory response and protecting epithelial cells from oxidative damage.^{2,27} In addition, this pattern correlates with more advanced healing, as groups with higher ERs also exhibited increased numbers of tissue fibroblasts, indicating a greater tendency to transition from the proliferative phase to the remodeling phase.²⁸

During the remodeling phase, wound tissue reorganizes and strengthens as collagen is produced and realigned, thereby increasing the wound's strength and elasticity.²⁵ The deposition of type I collagen, which confers greater resistance to scar tissue, was more prominent in the GLf and Fbn groups. In contrast, type III collagen, which predominates in the early phases of repair, was predominant in the Con group. The replacement of type III collagen by type I collagen is an important indicator of scar tissue maturation, suggesting that treatments with *L. ferrea*-based formulations contributed to and accelerated this process.^{29,30}

The wound-healing effects of *L. ferrea* on skin injuries in Wistar rats were investigated, supported by molecular docking analyses of its bioactive compounds. Gallic acid and ethyl gallate, known for their antioxidant, anti-inflammatory,^{31,32} and antimicrobial³³ properties, have been identified as key compounds that play a significant role in the wound healing process. These compounds demonstrated high binding affinity to the VEGFR-2/VEGF-A complex structure (PDB ID: 3V2A), with binding energies ranging from -5.5 to -5.6 kcal/mol.

It has been reported that topical application of gallic acid and ethyl gallate promotes healing by effectively modulating growth factors and cellular responses during the inflammation and proliferation phases of wound healing.³⁴ In this context, the VEGF, which promotes the formation of new blood vessels, plays a central role in wound healing.³⁵ VEGF activates three primary tyrosine kinase receptors—VEGFR-1, VEGFR-2, and VEGFR-3—to initiate angiogenic signaling, enhancing cellular angiogenic activity and supporting wound healing.³⁶

Moreover, the upregulation of hypoxia-inducible factor-1 α /VEGF/VEGFR-2 signaling has been reported to enhance angiogenic activity, thereby providing therapeutic effects in wound healing.³⁷ Binding ligands, such as gallic acid and ethyl gallate, trigger receptor dimerization, initiate transmembrane signaling, and activate receptor kinases, promoting angiogenesis and contributing to therapeutic effects in wound healing.³⁸

5. Conclusion

The integration of phytochemical data with histopathological results demonstrates that formulations containing high levels of tannins and flavonoids from *L. ferrea* fruits exert beneficial effects on wound healing by secondary intention, modulating inflammation, promoting granulation tissue formation, and accelerating epithelialization and collagen remodeling. The *in silico* study demonstrated that the main bioactive compounds of this species, gallic acid and ethyl gallate, contribute to the observed pharmacological effects by modulating vascular proliferation and fibroblast activity. These findings highlight the therapeutic potential of *L. ferrea*-based pharmaceutical formulations in wound treatment.

Acknowledgments

The authors would like to thank the Federal University of Amapá, through its Pro-Rectorate of Research and Postgraduate Studies, for support provided under the Publication Support Program.

Funding

This study was funded by the Coordination for the Improvement of Higher Education Personnel (*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*; CAPES) through the National Program for Academic Cooperation-Amazon Region (*Programa Nacional de Cooperação Acadêmica-Amazonia*; PROCAD Amazônia; 1723/2018-00 PROCAD-AMAZONIA) and by the National Council for Scientific and Technological Department (*Conselho Nacional de Desenvolvimento Científico e Tecnológico*; CNPq) through the Academic Master's and Doctoral Program for Innovation (*Programa de Mestrado e Doutorado Acadêmico para Inovação*; MAI-DAI; Proc. 421808/2022-5) and the National Institute of Science and Technology (INCT) North-Northeast Network of Phytoproducts (grant number: 421808/2022-5). This project received funding from the AmazonCure Company via the Priority Bioeconomy Program (PPBio - Priority Project: No. 06/2025), a public policy of the Superintendence of the Manaus Free Trade Zone (Suframa) coordinated by the Institute for Conservation and Sustainable Development of the Amazon (Idesam).

Conflict of interest

José Carlos Tavares Carvalho is an Editorial Board Member of this journal, but was not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. Separately, other authors declared that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Ethics approval and consent to participate

The animal study protocol was approved by the Research Ethics Committee of Federal University of Amapá under protocol no. 008/2017 of 08/23/2017.

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author on reasonable request.

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