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RESEARCH ARTICLE

Histopathological Evaluation and Computer-Aided Mechanistic Prediction of *Murraya koenigii* Extract on Excisional Wound Healing in Rats

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Abstract

Wound healing involves coordinated epithelial regeneration, extracellular matrix remodeling, and inflammatory regulation. This study evaluated the histopathological effects of topical *Murraya koenigii* leaf extract on excisional wound healing in rats and explored potential molecular mechanisms in silico. Fifty male Rattus norvegicus were divided into five groups (n=10 per group): unwounded control (CN), positive control (Bioplacenton®), and treatment groups 10%, 20%, and 40% *Murraya koenigii* ointment respectively. A standardized 1-cm full-thickness dorsal excisional wound was created, treatments were applied twice daily for 14 days, and tissues were collected on day 15. Sections were assessed using the SPOT scoring system (re-epithelialization, epithelial thickness index, keratinization, granulation thickness, remodeling, and scar elevation index). Kruskal-Wallis exhibited a significant difference in the composite SPOT score among groups ($H=40.838$, $P<0.001$) with a large effect ($\varepsilon^2=0.819$). Histopathologically, the 40% group showed the most promising repair, with more complete epithelial coverage, improved epidermal maturation, denser granulation tissue, and early dermal remodeling compared with lower concentrations. Molecular docking indicated favorable binding of major carbazole alkaloids (mahanine, mahanimbine, mahanimbicine) to FAAH and GSK3β, targets implicated in inflammation and regenerative signaling. These findings indicate that topical *Murraya koenigii* extract enhances structural wound repair and highlights its potential as a plant-based therapeutic candidate for wound management.

Keywords: Carbazole alkaloids, Endocannabinoid system, Wound repair, β-catenin signaling, Health, Phytotherapy

Introduction

Cutaneous wound healing is a highly regulated process with overlapping phases of hemostasis, inflammation, proliferation, and remodeling that restores tissue structure and function [1,2]. During proliferation, granulation tissue formation, angiogenesis, fibroblast proliferation, and re-epithelialization drive tissue restoration [3]. The rat excisional wound model provides standardized wound geometry and supports serial macro- and microscopic evaluation, including histopathological assessment of epithelial regeneration, inflammatory infiltration, and collagen deposition [4-6]. In

human studies, despite advances in wound care and topical therapeutics, delayed or impaired healing remains a clinical challenge, motivating continued exploration of bioactive compounds that modulate inflammation, oxidative stress, and extracellular matrix remodeling [7,8].

Natural products and plant-derived phytochemicals are increasingly studied for wound healing because of antioxidant, anti-inflammatory, and antimicrobial activities; among these, *Murraya koenigii* (curry leaf) is rich in carbazole alkaloids, flavonoids, and phenolic compounds with documented biological activities [9-12]. Formulations containing *Murraya koenigii* extract have been reported to enhance wound contraction, promote re-epithelialization, and support collagen synthesis *in vivo*, potentially via modulation of inflammatory mediators and oxidative-related pathways that influence tissue regeneration, matching trajectories observed in other therapeutic plant oil models [9,12-16].

However, although several studies have reported the wound-healing effects of *Murraya koenigii* based on macroscopic wound contraction and biochemical markers, systematic histopathological evaluation using standardized scoring systems remains limited [13,17]. Structured scoring approaches such as the SPOT system allow semi-quantitative assessment of epithelial regeneration, keratinization, granulation tissue maturation, and tissue remodeling. In addition to morphological assessment, *in silico* approaches such as molecular docking can provide mechanistic insights by predicting interactions between phytochemical compounds and proteins involved in inflammation, angiogenesis, and extracellular matrix regulation [18]. Integrating histopathological evaluation with computational analysis may therefore provide a more comprehensive understanding of the therapeutic potential of plant-derived compounds.

Therefore, the present study aimed to evaluate the effects of topical *Murraya koenigii* leaf extract on excisional wound healing in *Rattus norvegicus* using SPOT-based histopathological scoring and to explore potential molecular mechanisms through molecular docking analysis.

Material and Methods

Ethical Approval

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia (Approval No.: 1.KEH.043.03.2023), and conducted according to institutional animal care guidelines.

Study Design and Animals

A completely randomized design was used. Fifty male *Rattus norvegicus* (150-200 g; 3-4 months old) were acclimatized for one week and randomly assigned into five groups (n=10 per group): CN (truly unwounded and untreated control; these animals underwent no surgical procedures, anesthesia, or wound creation, and served as a histopathological benchmark for physiological normalcy), CP (Bioplacenton® ointment, Kalbe Farma, Indonesia serving as a widely used regional clinical standard for wound care that provides a benchmark for a successful healing trajectory), T1 (10% *Murraya koenigii),* T2 (20%), and T3 (40%). Each rat was considered one experimental unit. Group sizes (n=10 per group) and extract concentrations (10%, 20%, 40%) were selected based on established preclinical excisional wound-healing model parameters reported in the literature [4,5,19,20]. Treatments were applied topically twice daily for 14 consecutive days. Tissue samples were collected on day 15 for histopathological examination.

Animals were individually housed in polypropylene cages under controlled environmental conditions: ambient temperature 22±2°C, relative humidity 50-60%, and a 12-h light/dark cycle. All animals received a standard commercial pelleted rodent diet and had continuous access to clean water ad libitum throughout the study period. A mandatory one-week acclimatization period was observed prior to any experimental procedures, during which all animals were confirmed to be in good health before randomization.

Excisional Wound Model

The dorsal area of each rat was shaved and disinfected with 70% ethanol before surgery. Animals were anesthetized using ketamine (100 mg/kg) and xylazine (5 mg/kg) administered intraperitoneally. A circular full-thickness excisional wound with a diameter of 1 cm was created on the dorsal skin using a sterile biopsy punch. The wounds were left unsutured to allow secondary intentional healing. Postoperative assessment was monitored twice daily throughout the 14-day observation period using the Rat Grimace Scale (RGS), a validated objective tool for quantifying pain-related facial expressions in laboratory rodents [21]. Meloxicam (2 mg/kg, subcutaneous) was prepared as a rescue analgesic and designated for administration if any animal's RGS score exceeded the established clinical threshold for distress. Animals that exceeded the predetermined pain-score threshold received appropriate rescue analgesia to ensure animal welfare and alleviate pain-related distress. This reactive analgesic strategy was intentionally employed to balance ethical animal care with the need to minimize potential confounding effects of systemic non-steroidal anti-inflammatory drugs (NSAIDs) on the inflammatory and proliferative phases of wound healing, which were the primary histopathological outcomes assessed in the present study.

Preparation of *Murraya koenigii* Extract and Ointment

Cold dried curry leaves *(Murraya koenigii)* were macerated in 96% ethanol for 72 h at room temperature. The extract was filtered and concentrated under reduced pressure using a rotary evaporator at 40-45°C to obtain a viscous crude extract. The resulting extract was incorporated into a Vaselin flavum base to prepare ointments at concentrations of 10%, 20%, and 40% (total weight 50 g per formulation). All formulations were prepared under sterile conditions and stored in sealed containers until use *(Table 1)*.

Tissue Processing and Histopathology

Histopathological slides were independently evaluated by two blinded observers to minimize observer bias. Each SPOT parameter was assessed on a 0-2 ordinal scale (0 = absent/minimal; 1 = partial; 2 = complete/normal), following the criteria described by van de Vyver et al. [17]. Specifically: re-epithelialization scored the degree of continuous neo-epithelium coverage across the wound surface; the Epithelial Thickness Index (ETI) evaluated epidermal thickness relative to adjacent unwounded skin; keratinization assessed the organization of superficial keratin layers; granulation tissue maturation captured fibroblast density and early extracellular matrix deposition within the wound bed; remodeling evaluated collagen fibre organization and maturity; and the Scar Elevation Index (SEI) quantified epidermal architecture relative to the surrounding normal dermis. Interobserver agreement between the two blinded scorers was evaluated post hoc using Cohen's κ statistic. The analysis yielded a κ coefficient indicative of strong agreement across all six SPOT parameters, confirming the robustness of the semi-quantitative scoring. Any discrepancy between observers was resolved by consensus review.

In Silico Target Prediction and Molecular Docking

SMILES and three-dimensional structures of the ligands (mahanimbicine, mahanimbine, and mahanine) were obtained from PubChem. Potential protein targets were predicted using SwissTargetPrediction (species: *Rattus norvegicus)*. Protein sequences and structures were retrieved from the RCSB Protein Data Bank and UniProt databases. Sequence conservation between species was verified using Clustal Omega when necessary.

Table 1. *Composition of Murraya koenigii Ointment Formulations*

Group	Extract Concentration	*M. koenigii* Extract (g)	Vaselin Flavum (g)	Total Weight (g)
T1	10%	5	45	50
T2	20%	10	40	50
T3	40%	20	30	50

Ligand geometry optimization was performed using ORCA at the B3LYP/def2-SVP level with dispersion correction [22,23]. Protein and ligand preparation were carried out using BIOVIA Discovery Studio and ChimeraX. Docking validation was conducted by re-docking native inhibitors into the binding site to ensure reliability of the docking protocol, with acceptable root-mean-square deviation (RMSD ≤ 2 Å).

Molecular docking simulations were performed using AutoDock 4.2.6 with 100 genetic algorithm (GA) runs, a population size of 150, and 25×10^6 energy evaluations. Ligand-protein interactions were visualized using BIOVIA Discovery Studio [22].

Statistical Analysis

The data were analyzed using non-parametric statistical methods because the variables were ordinal. Differences among groups were evaluated using the Kruskal-Wallis test. When significant differences were detected, pairwise comparisons were further analyzed using the Mann-Whitney U test. To control the family-wise error rate, a Bonferroni correction was applied to all post-hoc pairwise comparisons. With ten possible pairwise comparisons among five experimental groups (C (5,2) = 10), the corrected significance threshold was adjusted to $\alpha = 0.005$ (0.05÷10). All pairwise differences reported as statistically significant met this corrected threshold.

Results

Histopathological Evaluation of Wound Healing

Histopathological examination performed on day 15 using the SPOT scoring system revealed significant differences among experimental groups across all evaluated parameters *(Table 2)*. Analysis using the Kruskal-Wallis test demonstrated statistically significant differences in scar elevation index (SEI), re-epithelialization, granulation tissue maturation, keratinization, epithelial thickness index (ETI), remodeling, and the total composite SPOT score ($P<0.05$ for all parameters).

Re-epithelialization differed significantly among groups ($H = 9.971$, $P=0.041$, $\varepsilon^2 = 0.133$). Pairwise comparisons using the Mann-Whitney U test revealed significant differences between CN and T1, CN and T2, as well as between T1 and T3 and T2 and T3. However, no significant difference was observed between T3 and the positive control group (CP) as well as T3 and the negative control group (CN). Histopathological sections demonstrated progressive epithelial bridging across the wound surface in the treated groups. The T3 group exhibited nearly complete epithelial coverage, indicating greater epidermal regeneration compared with the lower treatment concentrations *(Fig. 1)*.

Table 2. *Median and interquartile range (IQR) for each SPOT parameter across all experimental groups (CN, CP, T1, T2, T3), with overall Kruskal-Wallis H statistic presenting p-value, and ε^2 effect size*

Parameter	CN (n=10)	CP (n=10)	T1 10% (n=10)	T2 20% (n=10)	T3 40% (n=10)	H	p-value	ε^2
Re-epithelialization	2.0 [2.0-2.0]	2.0 [2.0-2.0]	2.0 [1.0-2.0]	2.0 [1.0-2.0]	2.0 [2.0-2.0]	9.971	0.041	0.133
Keratinization	2.0 [2.0-2.0]^a	2.0 [1.4-2.0]^a	1.2 [0.8-1.2]^b	2.0 [0.9-2.0]^a	2.0 [1.3-2.0]^a	20.966	0.000	0.377
ETI	2.0 [2.0-2.0]^a	2.0 [2.0-2.0]^a	1.0 [1.0-1.0]^b	2.0 [1.0-2.0]^a	2.0 [2.0-2.0]^a	27.601	0.000	0.524
Granulation	2.0 [2.0-2.0]^a	1.0 [1.0-2.0]^b	1.0 [1.0-1.0]^b	1.0 [0.3-1.0]^b	2.0 [2.0-2.0]^a	31.316	0.000	0.607
Remodeling	2.0 [2.0-2.0]^a	1.7 [1.2-2.0]^a	0.2 [0.1-0.4]^b	1.6 [1.1-2.0]^a	1.8 [1.5-2.0]^a	25.065	0.000	0.468
SEI	2.0 [2.0-2.0]^a	2.0 [2.0-2.0]^a	1.0 [0.25-2.0]^b	2.0 [1.0-2.0]^a	2.0 [2.0-2.0]^a	11.905	0.018	0.176
Total SPOT Score	12^a	10.7^a	6.4^b	10.6^a	11.8^a	40.838	0.000	0.819

CN = unwounded control; CP = positive control (Bioplacenton®); T1–T3 = 10%, 20%, 40% M. koenigii ointment; ETI = epithelial thickness index; SEI = scar elevation index.^{a,b} *values within a row with different superscript letters are significantly different (P<0.05) based on post-hoc pairwise comparisons*

Granulation tissue thickness demonstrated the most pronounced intergroup variation (H = 31.316, P<0.001, $\varepsilon^2 = 0.607$). The treated groups showed higher mean ranks compared with the control groups, indicating enhanced granulation tissue development. Histopathologically, this was characterized by greater fibroblast proliferation, increased vascularization, and early extracellular matrix deposition within the wound bed. These findings were most prominent in the T3 group, suggesting enhanced proliferative-phase activity.

Keratinization also differed significantly among groups (H = 20.966, P<0.001, ε^2 = 0.377). The T3 group demonstrated more organized keratin layers and improved epidermal maturation compared with T1 and T2. Similarly, the epithelial thickness index (ETI) showed significant variation (H = 27.601, P<0.001, ε^2 = 0.524). Groups treated with lower concentrations (T1 and T2) displayed increased epithelial thickness consistent with active epithelial proliferation, whereas the T3 group showed a more normalized epithelial architecture approaching that of the unwounded control.

Significant differences were also observed in the remodeling parameter (H = 25.065, P<0.001), reflecting variations in connective tissue organization and collagen maturation. Histopathological sections from the T3 group demonstrated early remodeling features, including more organized collagen fibers and reduced inflammatory infiltration compared with the lower-dose groups.

Importantly, the total composite SPOT score differed significantly among groups (H = 40.838, P<0.001, $\varepsilon^2 = 0.819$). The large effect size indicates that treatment with *Murraya koenigii* ointment accounted for a substantial proportion of the variation in overall histopathological healing outcomes. Mean rank comparisons further suggested a dose-dependent improvement in wound healing parameters, with the highest scores consistently observed in the T3 group. A comprehensive summary of the median and interquartile range (IQR) for each individual SPOT parameter across all

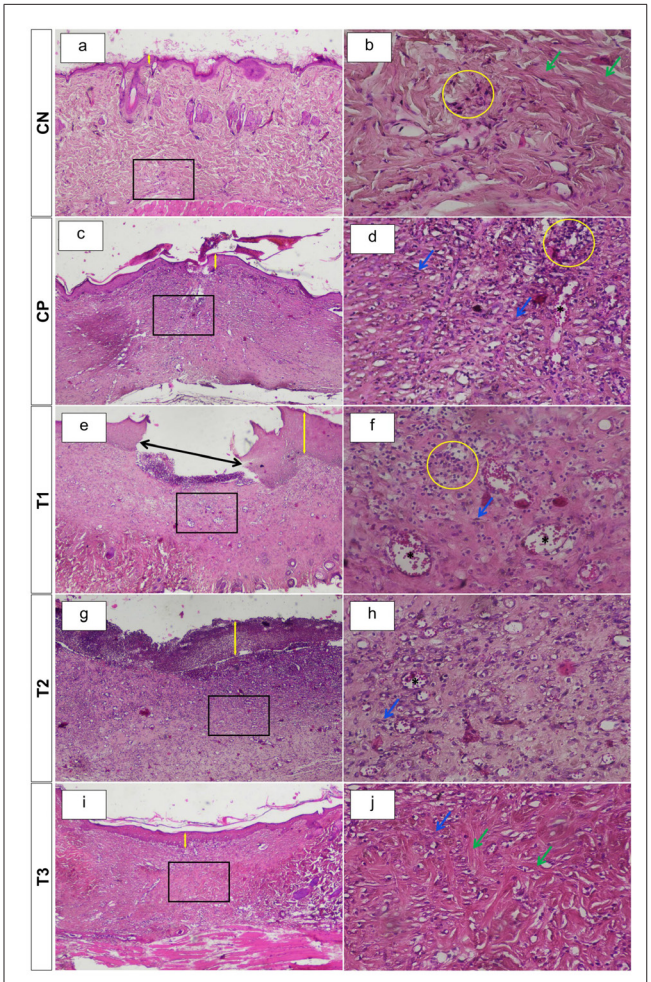

Fig 1. Histopathological features of experimental *Rattus norvegicus* skin tissue on day 15 of wound healing. Panels a, c, e, g, and i (H&E, 40×) show selected areas (*black boxes*), magnified in panels b, d, f, h, and j (H&E, 200×). Incomplete re-epithelialization (*black arrow*) was observed in T1. The CP and T3 groups showed thinner epithelial thickness (*yellow lines*) comparable to CN. Loose granulation tissue with angiogenesis, dilated blood vessels (*asterisks*), inflammatory infiltration (*yellow circles*), fibroblasts (*blue arrows*), and thin collagen fibers (*green arrows*) was evident in CP, T1, and T2. In contrast, T3 exhibited denser granulation tissue, fewer inflammatory cells, and thicker collagen fibers (*green arrows*), resembling CN

experimental groups, together with the Kruskal–Wallis H statistic, p-value, and ε^2 effect size, is presented in *Table 2*.

In Silico Mechanism Prediction

In silico target prediction identified fatty acid amide hydrolase (FAAH) and glycogen synthase kinase-3 beta (GSK3β) as potential molecular targets associated with epithelial regeneration and tissue repair. Docking validation was performed through re-docking of the native FAAH inhibitor into the active site, yielding a root-mean-square deviation (RMSD) value of 0.81 Å, which confirmed the reliability of the docking protocol *(Fig. 2)*. Sequence alignment analysis further supported the use of the human GSK3β structure as a surrogate model due to the high conservation of the catalytic binding pocket.

Molecular docking simulations demonstrated stable binding conformations for the carbazole alkaloids mahanimbicine, mahanimbine, and mahanine toward both target proteins. For FAAH, binding energies ranged from -9.69 to -10.16 kcal/mol, with mahanine exhibiting the strongest binding affinity (-10.16 kcal/mol) *(Fig. 2)*. For GSK3β, binding energies ranged from -8.41 to -8.95 kcal/mol, again with mahanine demonstrating the most favorable interaction (-8.95 kcal/mol) *(Fig. 3)*. To provide an overview of the experimental outcomes, the pathways linking the administration of mahanine, mahanimbine, and mahanimbicine to the observed changes in inflammatory markers and tissue regeneration are summarized in *Fig. 4*.

Visualization of ligand-protein interactions revealed several stabilizing contacts within the binding pocket, including hydrogen bonding, hydrophobic interactions, and van der Waals interactions. These interactions suggest potentially stable ligand-protein complexes that, if

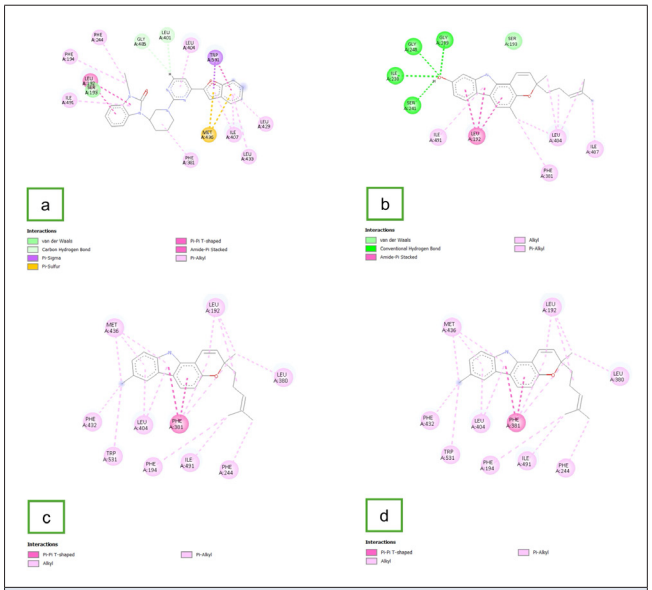

Fig 2. Molecular docking interaction diagrams illustrating the binding interactions of selected compounds from *Murraya koenigii* with the FAAH protein. Panels show the interaction profiles of **(a)** Native ligand QJ9, **(b)** mahanimbicine, **(c)** mahanimbine, and **(d)** mahanine within the FAAH active site, highlighting hydrogen bond and hydrophobic interactions with key amino acid residues

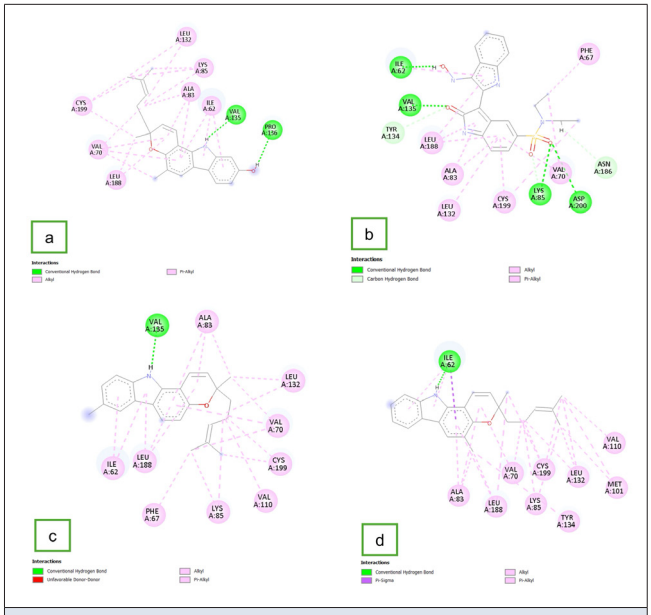

Fig 3. Molecular docking interaction diagrams of selected bioactive compounds from *Murraya koenigii* with the GSK3β protein. **(a)** Native ligand OFT, **(b)** mahanimbicine, **(c)** mahanimbine, and **(d)** mahanine docked within the GSK3β binding pocket, showing hydrogen bond and hydrophobic interactions with key amino acid residues

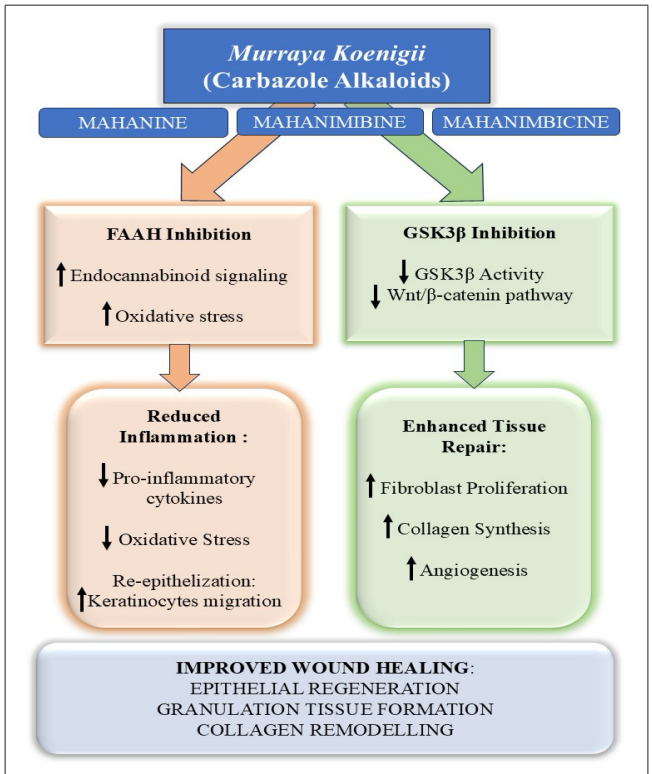

Fig 4. Schematic flowchart summarizing the mechanism of action of *Murraya koenigii* carbazole alkaloids in relation to inflammatory reduction and tissue repair markers

confirmed experimentally, may contribute to modulation of signaling pathways involved in inflammation and tissue regeneration.

Discussion

This study constitutes a foundational, exploratory investigation integrating standardized semi-quantitative histopathological scoring with *in silico* computational target prediction to characterize the wound-healing potential of topical *Murraya koenigii* extract in a rat excisional model. Cutaneous wound healing is a dynamic, multiphase process encompassing haemostasis, inflammation, proliferation, and remodeling, coordinated by keratinocytes, fibroblasts, endothelial cells, and immune effectors [1,3]. Successful repair requires re-epithelialization, angiogenesis-supported granulation tissue formation, progressive extracellular matrix remodeling, and epidermal maturation [24-26]. Plant-derived phytochemicals represent an actively investigated avenue for wound care, given their multi-target antioxidant, anti-inflammatory, and tissue-restorative activities [9,10,15]. Against this biological backdrop, the histopathological and computational findings of the present study are interpreted and contextualized below. It is important to emphasize that the *in silico* component of this work is intended as a hypothesis-generating tool to identify candidate molecular targets for future experimental investigation, and not as confirmation of pharmacological mechanism.

The overall histopathological evaluation demonstrated significant differences among experimental groups, with the Kruskal–Wallis test confirming that the total SPOT score differed across groups ($H = 40.838$, $P<0.001$), indicating that *Murraya koenigii* extract influenced wound healing [13,17,19,20]. The large effect size ($\varepsilon^2 = 0.819$) indicates that treatment explained a substantial proportion of variability in histopathological outcomes, suggesting a substantial treatment effect on histopathological wound-healing outcomes [1,3].

Consistent with SPOT-defined healing domains, group differences were expressed across re-epithelialization, granulation tissue formation, keratinization, fibroblast activity, and remodeling features [1,3,17,25,26]. Higher concentrations improved epidermal and dermal architecture. This was reflected by more complete epithelial coverage, increased fibroblast density, and denser collagen deposition. These findings are consistent with the proliferative and early remodeling phases of wound healing [25,26].

In addition, these findings align with previous studies highlighting the role of extracellular matrix components, particularly collagen and glycosaminoglycans such as hyaluronic acid (HA), in accelerating wound healing. HA is known to promote epithelial cell migration, proliferation, angiogenesis, and granulation tissue formation, especially during the early phases of repair. The observed increase in re-epithelialization, epidermal thickness, and collagen deposition in the treated groups may therefore reflect enhanced extracellular matrix dynamics and improved cellular activity, contributing to more efficient tissue regeneration [27,28].

Microscopically, treated groups demonstrated improved epithelial regeneration, as evidenced by partial to nearly complete epithelial coverage and increased epidermal thickness, indicating active keratinocyte-driven barrier restoration [29-32]. These findings indicate progression into the proliferative phase, characterized by re-epithelialization and tissue rebuilding, consistent with rat excisional wound models where topical agents such as resveratrol, ozonated olive oil, or insulin derivatives enhance epithelial regeneration and angiogenesis [33,34]. In parallel with these findings, the present study also showed more mature granulation tissue, reflected by increased fibroblast proliferation, neovascularization, and early collagen deposition, where these structural changes indicate active angiogenesis and extracellular matrix formation, both of which are essential for supporting tissue regeneration [17,25,27,35-37]. Similar histopathological improvements, including enhanced re-epithelialization, collagen deposition, and tissue organization, have been reported following topical treatment, likely mediated by moisture retention that preserves cellular viability, promotes angiogenesis, and enhances growth factor activity, thereby accelerating wound healing [35]. Taken together, these findings indicate that the enhanced histopathological features observed in the treated groups are not only consistent with previous experimental models but are also supported by underlying biological mechanisms that facilitate more efficient wound repair and tissue remodeling [24,26].

To explore plausible molecular mechanisms underlying these histopathological improvements, docking was performed against two repair-relevant targets associated with inflammation control and regenerative signaling: FAAH and GSK3β [22,23,29,36,37].

Regarding the specific interaction with FAAH, this enzyme degrades endocannabinoids (notably anandamide), and endocannabinoid system signaling has been linked with regulation of inflammation, nociception, and repair-associated cellular behavior; therefore, FAAH inhibition could potentially increase endocannabinoid signaling and contribute to a pro-healing wound microenvironment [29-31]. In the present docking results, *Murraya koenigii* carbazole alkaloids demonstrated favorable predicted binding affinity toward FAAH, with mahanine exhibiting the lowest binding energy (-10.16 kcal/mol), followed by mahanimbine (-9.70 kcal/mol) and mahanimbicine (-9.69 kcal/mol), suggesting

their potential as candidate FAAH inhibitors requiring further experimental confirmation [11,12,22,23,29]. If FAAH inhibition were experimentally confirmed, increased local endocannabinoid tone could plausibly attenuate excessive inflammation and support repair-associated cellular activities, including keratinocyte migration/proliferation and fibroblast-related extracellular matrix dynamics [29-31].

Parallel to the endocannabinoid pathway, the second computational target evaluated, GSK3β, serves as a key regulatory node in Wnt/β-catenin signalling, which can influence fibroblast activity, angiogenesis, and extracellular matrix dynamics during wound repair [36]. In addition, GSK3β-linked signalling has been implicated in keratinocyte migration and proliferation during re-epithelialization [37]. The carbazole alkaloids also exhibited favorable interactions with GSK3β (mahanine -8.95 kcal/mol; mahanimbine -8.85 kcal/mol; mahanimbicine -8.41 kcal/mol), suggesting potentially stable ligand-protein interactions [21,22]. Because GSK3β inhibition can stabilize β-catenin and activate Wnt signaling, such modulation may influence fibroblast activity, collagen deposition, and angiogenesis, aligning with the enhanced fibroblast density, granulation tissue organization, and extracellular matrix deposition in treated groups [36,37].

Overall, the observed wound-healing activity is consistent with evidence that plant-derived bioactive compounds and antioxidant-rich topical formulations can support repair through anti-inflammatory and tissue-restorative mechanisms, and prior work on *Murraya koenigii* also reports wound-healing relevant bioactivities, although many studies emphasize macroscopic contraction or general histopathological assessment rather than structured scoring [9-15,20,35]. By integrating SPOT-based histopathology with docking analysis, this study links structural repair outcomes to molecular targets involved in inflammation and regeneration [17,29,36,37].

Grounded in the integration of these histopathological observations and molecular docking predictions, a broader, integrated mechanistic model can be synthesized to describe the therapeutic activity of *Murraya koenigii*. It is critical to emphasize that the mechanistic narrative presented here is speculative and based exclusively on in silico computational predictions. No direct experimental confirmation of FAAH inhibition, GSK3β modulation, endocannabinoid elevation, or downstream Wnt/β-catenin pathway activation has been performed within the scope of this study. The proposed model should therefore be interpreted solely as a predictive roadmap to guide future wet-laboratory validation (e.g., Western blot, qPCR, immunohistochemistry, enzymatic activity assays) and not as evidence of confirmed pharmacological mechanism. Causal relationships between the carbazole alkaloids and the observed histopathological improvements cannot be established from the present data alone.

Based on the integration of histopathological observations and molecular docking predictions, a potential mechanistic model can be proposed to explain the wound-healing activity of *Murraya koenigii*. Although experimental validation is required, the carbazole alkaloids present in the extract may simultaneously modulate multiple molecular pathways involved in tissue repair.

First, inhibition of FAAH may increase endocannabinoid levels in the wound microenvironment, thereby reducing inflammatory responses and promoting keratinocyte migration, which accelerates re-epithelialization [26-30]. Second, modulation of GSK3β activity may enhance wound repair by promoting keratinocyte EMT, migration, and proliferation to accelerate re-epithelialization; rFGF4 treatment has also been associated with increased collagen deposition and vascular markers *in vivo* [14,33,35]. These processes facilitate granulation tissue formation, extracellular matrix deposition, and tissue remodelling during the proliferative phase of wound healing [20,24,25]. The combined modulation of these pathways may create a favourable microenvironment for tissue regeneration, resulting in improved histopathological outcomes such as enhanced epithelial coverage, increased granulation tissue organization, and early epidermal maturation [19,25,32,33].

Overall, these findings suggest that the carbazole alkaloids present in *Murraya koenigii* are predicted to exert complementary modulatory effects that promote wound healing through both anti-inflammatory mechanisms and stimulation of tissue regeneration *(Fig. 4)*. The integration of *in vivo* histopathological evaluation and *in silico* molecular docking analysis therefore provides complementary evidence supporting the therapeutic potential of *Murraya koenigii* as a candidate for wound-healing applications. Nevertheless, several important limitations temper these conclusions and are addressed comprehensively further.

First, histopathological assessment was conducted only at day 15; therefore, temporal changes during the early inflammatory and intermediate proliferative phases could not be characterized. Future studies should incorporate multiple sampling time points to capture the complete wound-healing trajectory. Second, the proposed involvement of FAAH and GSK3β was inferred from molecular docking analyses without experimental validation. Future investigations should employ molecular and biochemical approaches, such as gene and protein expression analyses, to confirm target engagement and elucidate underlying mechanisms. Third, a wounded untreated control group was not included. Consequently, the therapeutic effect of the treatment could not be directly compared with the natural healing process. Inclusion of a spontaneous-healing control group would allow more accurate estimation of treatment efficacy.

Fourth, the contribution of the vehicle could not be independently evaluated because a vehicle-only control group was not included. Future studies should incorporate a petrolatum-only treatment arm to distinguish vehicle-related effects from those of the plant extract. Fifth, phytochemical standardization of the extract was not performed, limiting reproducibility and precise dose–response interpretation. Quantitative characterization of major bioactive constituents using chromatographic techniques is therefore warranted. Finally, functional and biochemical wound-healing outcomes, including wound contraction, tensile strength, oxidative stress markers, and inflammatory mediators, were not assessed. Integrating these endpoints in future studies would provide a more comprehensive evaluation of therapeutic efficacy and mechanism of action.

Despite these limitations, the present study provides preliminary evidence supporting the wound-healing potential of *Murraya koenigii* and identifies FAAH and GSK3β as promising targets for future mechanistic investigation.

Declarations

Availability of Data and Materials: The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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Author Contributions: T.S.S. and L.R.Y. conceived and designed the study. Z.T. conducted the molecular docking analysis. E.A.b.A., S.V.J., and Y.I.K. performed the experimental procedures and primary data collection. R.K.D. and R.S. contributed to extract preparation and formulation development. L.R.Y., A.M., and E.P.H. conducted the histopathological evaluation, microscopic imaging, and data interpretation. D.N.I., M.N.R., and K.P.S. performed the statistical analysis. T.S.S., Y.I.K., and L.R.Y. drafted and reviewed the manuscript. All authors critically revised the manuscript and approved the final version for publication.

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