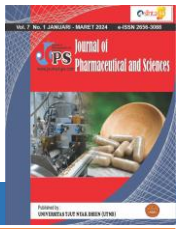
Journal of Pharmaceutical and Sciences
Electronic ISSN: 2656-3088 DOI: https://doi.org/10.36490/journal-jps.com
Homepage: https://journal-jps.com
ORIGINAL ARTICLE JPS. 2026, 9(2), 1835-1847



Formulation and Evaluation of Aloe vera–Andaliman (*Zanthoxylum acanthopodium*) Gel for Epidermolysis Bullosa Wound Healing: Phytochemical, In Silico, and In Vivo Approaches

Formulasi dan Evaluasi Gel Aloe vera–Andaliman (*Zanthoxylum acanthopodium*) untuk Penyembuhan Luka Epidermolisis Bulosa: Pendekatan Fitokimia, *In Silico*, dan *In Vivo*

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Abstract
Epidermolysis Bullosa (EB) is a rare genetic skin disorder characterized by chronic wounds and delayed healing, requiring alternative therapies that support tissue regeneration. This study aimed to develop and evaluate a gel formulation containing Aloe vera extract and Andaliman (*Zanthoxylum acanthopodium*) essential oil for EB wound healing. The novelty of this study lies in the combination of Aloe vera and Andaliman as a topical phytopharmaceutical approach for EB-like wounds. This combination was selected based on the complementary mechanisms of Aloe vera, which supports wound healing, fibroblast activity, and re-epithelialization, and Andaliman, which exhibits antioxidant, antimicrobial, and anti-inflammatory properties. Phytochemical screening, GC–MS analysis, antioxidant assay, gel evaluation, in silico docking against VEGF-A, and in vivo testing in EB-like wound rats were performed. Phytochemical screening revealed alkaloids, flavonoids, saponins, tannins, steroids, and glycosides. GC–MS identified 38 compounds in Aloe vera and 20 compounds in Andaliman. Antioxidant testing showed IC50 values of 165.2 µg/mL for Aloe vera and 98.7 µg/mL for Andaliman. The gel formulations showed acceptable physicochemical properties, with pH 5.8–6.1, viscosity 3,800–4,700 cPs, and good adhesion. Docking results showed strong interactions of lupeol, anthranol, and paulownin with VEGF-A. In vivo, F3 (4% Aloe vera and 3% Andaliman) accelerated wound closure, improved epithelialization, reduced inflammatory infiltration, and significantly increased VEGF expression ($p < 0.0001$). These findings suggest that Aloe vera–Andaliman gel is a promising topical candidate for EB wound management.

***Keywords*: Aloe vera, Andaliman, gel formulation, wound healing, epidermolysis bullosa, VEGF**

Abstrak
Epidermolisis Bulosa (EB) merupakan kelainan kulit genetik langka yang ditandai dengan luka kronis dan keterlambatan penyembuhan, sehingga membutuhkan terapi alternatif yang mendukung regenerasi jaringan. Penelitian ini bertujuan untuk mengembangkan dan mengevaluasi formulasi gel yang mengandung ekstrak Aloe vera dan minyak atsiri Andaliman (*Zanthoxylum acanthopodium*) untuk penyembuhan luka EB. Kebaruan penelitian ini terletak pada kombinasi Aloe vera dan Andaliman sebagai pendekatan fitofarmaka topikal untuk luka menyerupai EB. Kombinasi ini dipilih berdasarkan mekanisme yang saling melengkapi, yaitu Aloe vera yang mendukung penyembuhan luka, aktivitas fibroblas, dan re-epitelisasi, serta Andaliman yang memiliki aktivitas antioksidan, antimikroba, dan antiinflamasi. Penelitian ini meliputi skrining fitokimia, analisis GC–MS, uji antioksidan, evaluasi gel, docking in silico terhadap VEGF-A, dan uji in vivo pada tikus dengan luka menyerupai EB. Skrining fitokimia menunjukkan adanya alkaloid, flavonoid, saponin, tanin, steroid, dan glikosida. Analisis GC–MS mengidentifikasi 38 senyawa pada Aloe vera dan 20 senyawa pada Andaliman. Uji antioksidan menunjukkan nilai IC50 sebesar 165,2 µg/mL untuk Aloe vera dan 98,7 µg/mL untuk Andaliman. Formulasi gel menunjukkan sifat fisikokimia yang baik, dengan pH 5,8–6,1, viskositas 3.800–4.700 cPs, dan daya lekat yang baik. Hasil docking menunjukkan interaksi kuat lupeol, anthranol, dan paulownin dengan VEGF-A. Secara in vivo, F3 (4% Aloe vera dan 3% Andaliman) mempercepat penutupan luka, meningkatkan epitelisasi, menurunkan infiltrasi inflamasi, dan meningkatkan ekspresi VEGF secara signifikan ($p < 0,0001$). Temuan ini menunjukkan bahwa gel Aloe vera–Andaliman berpotensi sebagai kandidat topikal untuk penatalaksanaan luka EB.

***Kata Kunci:** Aloe vera, andaliman, formulasi gel, penyembuhan luka, epidermolisis bulosa, VEGF.*

https://doi.org/10.36490/journal-jps.com.v9i2.1646

Article History:
Received: 13/04/2026,
Revised: 22/06/2026
Accepted: 22/06/2026,
Available Online: 23/06/2026.

QR access this Article

Introduction

Epidermolysis Bullosa (EB) is a rare genetic disorder characterized by extreme fragility of the skin and mucous membranes, resulting in chronic wounds, blister formation, and recurrent infections [1]. Patients with EB experience significant morbidity, including persistent pain, limited mobility, malnutrition, and psychological distress [2]. Current management strategies remain largely palliative, focusing on wound dressing, infection prevention, nutritional support, and pain control, while curative options remain limited [3]. Therefore, there is a growing need for safe and effective topical agents that can promote wound closure, reduce inflammation, and enhance tissue regeneration in chronic wound conditions such as EB.

Natural products have gained increasing attention in wound management due to their multi-target pharmacological activities, biocompatibility, and relatively low toxicity. Among them, Aloe vera is one of the most extensively studied medicinal plants traditionally used for skin disorders, burns, and wound healing [4]. Aloe vera gel contains anthraquinones, flavonoids, saponins, glycosides, sterols, and polysaccharides that contribute to antioxidant, antimicrobial, anti-inflammatory, and wound healing activities [5]. These bioactive compounds may support fibroblast proliferation, collagen synthesis, re-epithelialization, and angiogenesis, which are essential processes in tissue repair.

In addition to Aloe vera, Andaliman (*Zanthoxylum acanthopodium*), an indigenous spice from North Sumatra, has been reported to contain volatile oils, lignans, sterols, and other bioactive constituents with antioxidant, antimicrobial, and anti-inflammatory properties [6]. Compounds such as fargesin, paulownin, and phytosterols may contribute to the modulation of inflammatory pathways and protection against oxidative damage [7,8]. However, scientific evidence regarding the wound healing potential of Andaliman remains limited, particularly in combination with other botanical agents such as Aloe vera.

Formulating a topical gel that combines Aloe vera extract and Andaliman essential oil may provide a promising strategy for EB wound management. Gel formulations are advantageous for wound treatment because they can maintain a moist environment, improve patient comfort, prolong contact time at the wound surface, and enhance the delivery of bioactive compounds [9]. Considering the chronic and recurrent nature of EB wounds, a topical formulation with antioxidant, anti-inflammatory, antimicrobial, and pro-regenerative properties may offer complementary benefits. EB is a heterogeneous inherited skin disorder, and preclinical wound models remain useful for evaluating topical candidates that may improve wound repair and tissue regeneration [10].

Angiogenesis is one of the key mechanisms required for effective wound healing, as it supports oxygen and nutrient delivery to regenerating tissue. Vascular endothelial growth factor A (VEGF-A) plays a central role in angiogenesis by promoting endothelial cell proliferation and new capillary formation [11]. Therefore, evaluating the interaction between selected phytochemicals and VEGF-A may provide mechanistic insight into the potential pro-angiogenic activity of the formulation.

The present study aimed to formulate and evaluate a topical gel containing Aloe vera extract and Andaliman essential oil for EB-like wound healing. This study employed an integrated approach consisting of phytochemical screening, GC–MS analysis, antioxidant assay, physicochemical evaluation of gel formulations, in silico molecular docking against VEGF-A, and in vivo wound healing assessment in an EB-like rat model. This approach is expected to provide scientific evidence for the development of Aloe vera–Andaliman gel as a potential phytopharmaceutical candidate for chronic wound management.

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Materials and Methods

Plant Material and Extraction

Fresh Aloe vera leaves were obtained from a local cultivation area in North Sumatra, Indonesia. The plant material was identified and authenticated by UPT Laboratorium Herbal Materia Medica Batu, Batu, East Java, Indonesia, under determination certificate number 00.9.3/0375/102.20/2025. The leaves were washed with running water, cleaned from impurities, and processed immediately to obtain the inner gel.

Andaliman fruits (*Zanthoxylum acanthopodium*) were obtained from North Sumatra, Indonesia. The plant material was identified and authenticated by UPT Laboratorium Herbal Materia Medica Batu, Batu, East Java, Indonesia, under determination certificate number 00.9.3/0376/102.20/2025. The fruits were cleaned, air-dried at room temperature, and stored in tightly closed containers protected from direct light and humidity until hydrodistillation. The fruits were cleaned, air-dried at room temperature, and stored in tightly closed containers protected from direct light and humidity until hydrodistillation.

Phytochemical Screening

Qualitative phytochemical screening of Aloe vera extract was performed using standard protocols for alkaloids (Mayer's, Wagner's, and Bouchardat reagents), flavonoids (Shinoda test), saponins (frothing test), tannins ($FeCl_3$ test), steroids/triterpenoids (Liebermann–Burchard test), and glycosides (Keller–Killiani test) [12].

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

Chemical profiling of Aloe vera extract and Andaliman essential oil was carried out using GC–MS (Agilent Technologies). Separation was performed on an HP-5MS capillary column, with helium as carrier gas at 1 mL/min. The oven temperature was programmed from 50 °C to 280 °C at 10 °C/min. Compounds were identified by comparison with the NIST library database [13].

Antioxidant Activity Assay

The antioxidant activity of Aloe vera extract and Andaliman essential oil was evaluated using the DPPH free radical scavenging assay. Each sample was dissolved in ethanol to prepare a stock solution of 1000 µg/mL. Serial dilutions were then prepared at concentrations of 2, 4, 8, 16, 32, 64, 128, 256, and 400 µg/mL. Quercetin was used as the positive control. A 0.1 mM DPPH solution was prepared in ethanol. An equal volume of DPPH solution and each sample dilution was mixed and incubated at room temperature for 30 minutes in the dark. The absorbance was measured at 517 nm using a UV–Vis spectrophotometer. The percentage of radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

where Acontrol is the absorbance of DPPH solution without sample, and Asample is the absorbance of DPPH solution with sample. The IC_{50} value, defined as the concentration required to inhibit 50% of DPPH radicals, was calculated using linear regression analysis of percentage inhibition against log concentration. All measurements were performed in triplicate, and the IC_{50} values were expressed as mean ± SD[14].

Gel Formulation

The concentration ranges of Aloe vera extract (2–4%) and Andaliman essential oil (1–3%) were selected based on preliminary formulation compatibility studies, dose-response considerations, and previous reports on the wound healing potential of Aloe vera- and Andaliman-based topical preparations. Aloe vera has been widely reported to support wound healing, re-epithelialization, and tissue repair, while Andaliman has shown anti-inflammatory and wound healing-related activity in topical preparations [6]. In the preliminary formulation stage, these concentration ranges produced homogeneous gels with acceptable pH, viscosity, spreadability, and adhesion, without visible signs of phase separation or instability. Therefore, F1–F3 were prepared to evaluate whether increasing concentrations of both active ingredients could improve wound healing responses while maintaining acceptable physicochemical properties.

Evaluation of Gel Formulations

The prepared gel formulations were subjected to a series of physicochemical evaluations to ensure their quality and stability. Organoleptic properties, including color, odor, and homogeneity, were first observed to

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confirm acceptable appearance and consistency. The pH of each formulation was measured with a calibrated digital pH meter on day 0 and day 14 to verify compatibility with the physiological range of skin. Viscosity was determined using a Brookfield viscometer (spindle no. 64, 20 rpm) to assess the consistency and ease of application. Spreadability was evaluated by measuring the diameter of gel spread under a 50 g load, while adhesion was determined as the time required for gel detachment under the same load. Stability testing was performed by storing the formulations at room temperature for 14 days and observing any physical changes. These evaluations provided critical information on the suitability of the gels for topical wound healing applications [15,16].

In Silico Docking

Bioactive compounds identified by GC–MS were docked against VEGF-A protein (PDB ID: 6ZFL) using AutoDock Vina. Protein structures were prepared by removing water molecules and adding hydrogen atoms. Ligand structures were retrieved from PubChem and energy-minimized. Docking scores (kcal/mol) were recorded, and 2D interactions were visualized using Discovery Studio [17].

In Vivo EB-like Superficial Abrasion Wound Model

The wound healing activity of the gel formulations was evaluated using an EB-like superficial abrasion wound model in male Wistar rats. The term "EB-like" was used because the model was designed to mimic superficial skin fragility and abrasion-like lesions observed in EB-related wounds, rather than to reproduce the genetic defects underlying EB. Before wound induction, the dorsal hair of each rat was carefully shaved and the skin surface was cleaned. Superficial abrasions were then produced on the dorsal skin using a sterile abrasive instrument until mild epidermal disruption and erythema were observed, without creating a full-thickness excision wound. The wound margins in this model were irregular and superficial; therefore, wound diameter or wound area was not measured quantitatively. Macroscopic wound healing was evaluated descriptively based on visible changes, including erythema, inflammation, exudate, granulation tissue formation, epithelialization, and wound closure [18].

In Vivo Wound Healing Study

Male Wistar rats (200–250 g) were used and divided into five groups: normal, placebo (gel base only), and treatment groups (F1–F3). EB-like wounds were induced by mechanical abrasion. Treatments were applied topically once daily for 10 days. Wound healing was monitored macroscopically (days 1, 4, 7, 10), and tissues were harvested for histological analysis (H&E staining).

VEGF Expression Assay

VEGF levels in wound tissue homogenates were measured using ELISA kits according to manufacturer instructions. Data were expressed as pg/mL.

Statistical Analysis

Statistical analysis was performed only for measurable quantitative parameters, particularly VEGF expression levels, using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value of less than 0.05 was considered statistically significant.

Results and Discussion

Phytochemical screening of Aloe vera extract showed the presence of alkaloids, saponins, tannins, flavonoids, steroids/triterpenoids, and glycosides (Table 1). In the alkaloid test, positive reactions were observed with Mayer's and Dragendorff's reagents, while Bouchardat's reagent showed a negative result. This discrepancy may be related to differences in reagent sensitivity, the low concentration of certain alkaloid subtypes, or possible interference from other compounds in the extract. Therefore, the alkaloid result was interpreted as positive based on the positive reactions obtained from two alkaloid-specific reagents.These bioactive compounds are widely associated with wound healing activity, where flavonoids provide antioxidant protection, saponins act as antimicrobials and membrane stabilizers, tannins enhance wound contraction, and steroids reduce inflammation [19]. The finding corresponds with earlier reports that *Aloe vera* is a rich source of therapeutic phytochemicals capable of accelerating tissue regeneration.

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Table 1. Phytochemical screening of Aloe vera extract

No.	Secondary metabolite	Test/reagent	Result
1	Alkaloids	Mayer's reagent	Positive
		Dragendorff's reagent	Positive
		Bouchardat's reagent	Negative
2	Saponins	Foam test	Positive
3	Tannins	$FeCl_3$ test	Positive
4	Flavonoids	Shinoda test	Positive
5	Steroids/triterpenoids	Liebermann–Burchard test	Positive
6	Glycosides	Keller–Killiani test	Positive

Table 2. GC–MS analysis of Aloe vera extract

No	R.Time (min)	Area (%)	Compound Identified
1	2.047	3.14	L-Arabinopyranose
2	2.240	1.25	D-Glucopyranose
3	2.292	3.87	D-Mannopyranose
4	2.662	0.60	Rhamnose
5	3.067	0.69	Sorbitol
6	3.709	1.49	2-Hydroxy-1,2,3-propane-tricarboxylic acid
7	3.995	0.33	Citric acid
8	4.200	0.36	Succinic acid
9	4.536	0.36	Aloe-emodin
10	4.884	0.07	Aloin A
11	5.118	0.13	Aloin B
12	5.573	0.89	1,8-Dihydroxy-3-methyl-anthraquinone
13	6.693	31.41	Aloe-emodin anthrone
14	8.204	0.10	Aloesin
15	8.609	0.22	Aloeresin B
16	8.771	2.62	Isobarbaloin
17	9.094	3.64	Barbaloin
18	9.595	0.08	Aloeresin C
19	10.150	13.41	Aloeresin D
20	10.567	23.11	Anthranol
21	10.924	0.25	24-Methylene-cycloartanol
22	12.537	0.14	24-Ethyl-lophenol
23	13.198	0.07	Lupeol
24	13.826	0.36	β-Sitosterol
25	15.873	0.79	Campesterol
26	16.091	0.35	Stigmasterol
27	16.391	0.80	Cycloartenol
28	17.033	1.03	Tocopherol
29	17.275	2.04	Hexadecanoic acid
30	17.477	0.85	Octadecanoic acid
31	17.839	0.67	cis-9-Octadecenoic acid
32	18.566	1.16	Linoleic acid
33	18.733	0.43	α-Linolenic acid
34	19.001	0.24	Arachidic acid
35	20.349	0.58	3,7,11,15-Tetramethylhexadec-2-en-1-ol
36	21.061	0.69	Octadecanoic acid ester
37	21.424	1.63	Hexadecanoic acid
38	22.478	0.57	Ursolic acid

Gas chromatography–mass spectrometry (GC–MS) confirmed the presence of 38 compounds in *Aloe vera* and 20 compounds in Andaliman essential oil. In *Aloe vera*, the dominant components were Aloe-emodin anthrone (31.41%), Anthranol (23.11%), and Aloeresin D (13.41%) (Table 2). Andaliman oil was also dominated

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by Aloe-emodin anthrone (31.41%) and Anthranol (23.11%) (Table 3). Both extracts contained sterols (β-sitosterol, lupeol) and fatty acids (linoleic, α-linolenic) that contribute to antioxidant and anti-inflammatory properties. The GC–MS analysis showed that Aloe vera extract and Andaliman essential oil shared several similar compounds, including Aloe-emodin anthrone and Anthranol. This finding should be interpreted with caution because compound identification was based on GC–MS library matching, which may involve analytical overlap, co-elution, or similar fragmentation patterns. However, each sample also showed distinct phytochemical profiles, indicating that Aloe vera and Andaliman may contribute different bioactive components to the combination gel. Further confirmation using authentic standards or complementary methods such as HPLC or LC–MS/MS is recommended to validate the identity of these overlapping compounds. The presence of similar anthraquinone compounds in both extracts is noteworthy and may indicate either shared biosynthetic pathways or potential analytical overlap. However, the distinct compound profiles of each extract suggest that each contributes unique bioactive components to the combination formulation

Table 3. GC–MS analysis of Andaliman essential oil

No	R.Time (min)	Area (%)	Compound Identified
1	10.567	23.11	Anthranol
2	6.693	31.41	Aloe-emodin anthrone
3	18.566	1.16	Linoleic acid
4	9.094	3.64	Barbaloin
5	3.709	1.49	2-Hydroxy-1,2,3-propane-tricarboxylic acid
6	13.826	0.36	β-Sitosterol
7	2.240	1.25	D-Glucopyranose
8	22.478	0.57	Ursolic acid
9	4.536	0.36	Aloe-emodin
10	13.198	0.07	Lupeol
11	8.771	2.62	Isobarbaloin
12	17.275	2.04	Hexadecanoic acid
13	2.292	3.87	D-Mannopyranose
14	16.391	0.80	Cycloartenol
15	5.573	0.89	1,8-Dihydroxy-3-methyl-anthraquinone
16	17.839	0.67	cis-9-Octadecenoic acid
17	15.873	0.79	Campesterol
18	2.047	3.14	L-Arabinopyranose
19	8.609	0.22	Aloeresin B
20	17.477	0.85	Octadecanoic acid

Table 4. IC_{50} Values of Aloe vera Extract, Andaliman Oil, and Quercetin as a Reference Standard in the Antioxidant Assay

Sample	IC_{50} (μg/mL)
Aloe vera extract	165.2
Andaliman oil	98.7
Quercetin standard	5.57

The antioxidant assay showed that Aloe vera extract and Andaliman essential oil exhibited IC_{50} values of 165.2 ± 7.4 μg/mL and 98.7 ± 4.6 μg/mL, respectively, while quercetin showed stronger antioxidant activity with an IC_{50} value of 5.57 ± 0.28 μg/mL. These results indicate that both natural samples exhibited moderate antioxidant activity compared with the pure standard compound. This finding is reasonable because crude extracts and essential oils contain complex mixtures of compounds, and their biological effects are not determined solely by direct radical scavenging capacity.

Although the antioxidant activity was moderate, it remains therapeutically relevant for wound healing. Chronic wounds, including EB-like lesions, are associated with persistent oxidative stress that can impair fibroblast activity, collagen deposition, angiogenesis, and re-epithelialization. In this context, moderate and sustained antioxidant activity may help reduce excessive reactive oxygen species without completely suppressing redox signaling, which is also required for normal tissue repair. Therefore, the antioxidant

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properties of Aloe vera and Andaliman may contribute to the wound healing process as part of a broader mechanism involving anti-inflammatory, antimicrobial, and pro-angiogenic effects.Three gel formulations were successfully prepared (Table 5). All exhibited clear, homogeneous appearances and remained stable for 14 days (Figure 1). Their pH values were within the safe range for topical application (5.8–6.1). With increasing polymer and active content, viscosity rose from 3,800 to 4,700 cPs, reducing spreadability but improving adhesion (Table 6). Longer adhesion times are favorable for topical wound therapy, as they prolong contact between actives and wound surfaces.

Table 5. Gel formulation composition

Component (% b/b)	F1	F2	F3
Aloe vera extract	2	3	4
Andaliman oil	1	2	3
HPMC	1	2	3
Propylene glycol	15	15	15
Methylparaben	0.075	0.075	0.075
Propylparaben	0.025	0.025	0.025
Aqua	ad 100	ad 100	ad 100

Table 6. Physicochemical evaluation of Aloe vera–Andaliman gel formulations

Parameter	F1	F2	F3
Organoleptic	Clear, homogeneous	Clear, viscous	More viscous, homogeneous
pH (day 0)	6.1 ± 0.1	6.0 ± 0.1	5.9 ± 0.1
pH (day 14)	6.0 ± 0.1	5.9 ± 0.1	5.8 ± 0.1
Spreadability (cm, 50 g)	5.8 ± 0.2	5.4 ± 0.1	4.9 ± 0.2
Viscosity (cPs)	3,800 ± 50	4,200 ± 60	4,700 ± 70
Adhesion (s)	35 ± 2	42 ± 3	51 ± 3
Stability	Stable	Stable	Stable

Although the 14-day stability evaluation showed acceptable short-term physical stability of the gel formulations, this period is insufficient to establish the long-term stability and shelf life of the product. Therefore, further stability studies under both real-time and accelerated conditions are required. Future studies should include stability testing at 25°C/60% RH and 40°C/75% RH for at least 6 months, with periodic evaluation of organoleptic properties, homogeneity, pH, viscosity, spreadability, adhesion, and active compound content.

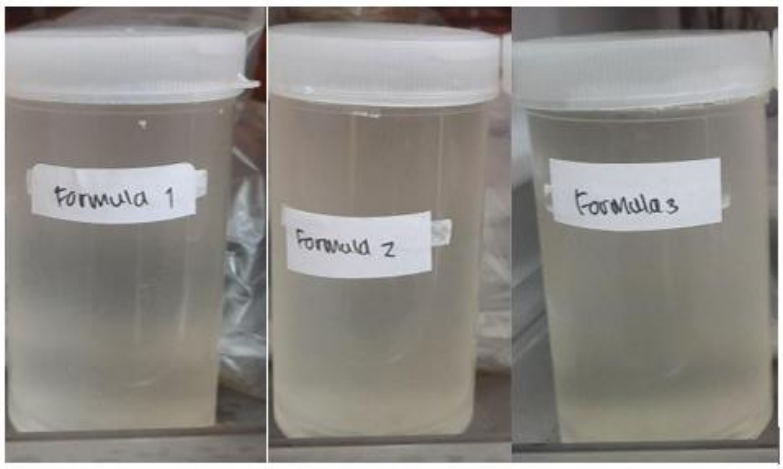

Figure 1. Representative images of Aloe vera–Andaliman gels (F1–F3).

Molecular docking was performed to evaluate the potential interaction between selected bioactive compounds and VEGF-A. In docking interpretation, a more negative binding affinity value indicates a more stable ligand–protein interaction. In general, binding affinity values below −7.0 kcal/mol are considered to indicate strong interactions, while values between −5.0 and −7.0 kcal/mol suggest moderate interactions. Based

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on this criterion, lupeol (−7.4 kcal/mol), anthranol (−7.1 kcal/mol), fargesin (−7.1 kcal/mol), and paulownin (−8.1 kcal/mol) demonstrated strong potential interactions with VEGF-A. These results suggest that selected compounds from Aloe vera and Andaliman may contribute to wound healing through modulation of angiogenesis-related pathways.

These results support earlier studies that triterpenes and lignans enhance VEGF signaling, thereby stimulating vascularization and tissue repair [20]. VEGF (vascular endothelial growth factor) plays a central role in wound repair by promoting angiogenesis, endothelial cell proliferation, and new capillary formation at the wound site. The stimulation of VEGF pathways accelerates oxygen and nutrient delivery to regenerating tissues, which is critical in chronic wounds such as EB. Previous studies have shown that natural compounds like lupeol can activate VEGF expression and support fibroblast proliferation [21], while anthraquinones such as anthranol act as modulators of oxidative stress and inflammation, indirectly supporting angiogenesis [22]. Similarly, lignans have been documented to exhibit anti-inflammatory and antioxidant activities that synergize with angiogenic pathways [23].

Thus, the docking results not only provide molecular evidence of Aloe vera–Andaliman compounds interacting with VEGF-A but also align with established pharmacological theories that phytochemicals can accelerate wound healing through simultaneous modulation of oxidative stress, inflammation, and angiogenesis.

Table 7. Docking results of Aloe vera compounds with VEGF-A

Compound	Binding Affinity (kcal/mol)
Aloe-emodin	−6.8
Barbaloin	−6.8
Anthranol	−7.1
Ursolic acid	−6.9
Lupeol	−7.4

Table 8. Docking results of Andaliman compounds with VEGF-A

Compound	Binding Affinity (kcal/mol)
Phytol	−4.8
Vitamin E	−6.2
Fargesin	−7.1
Paulownin	−8.1

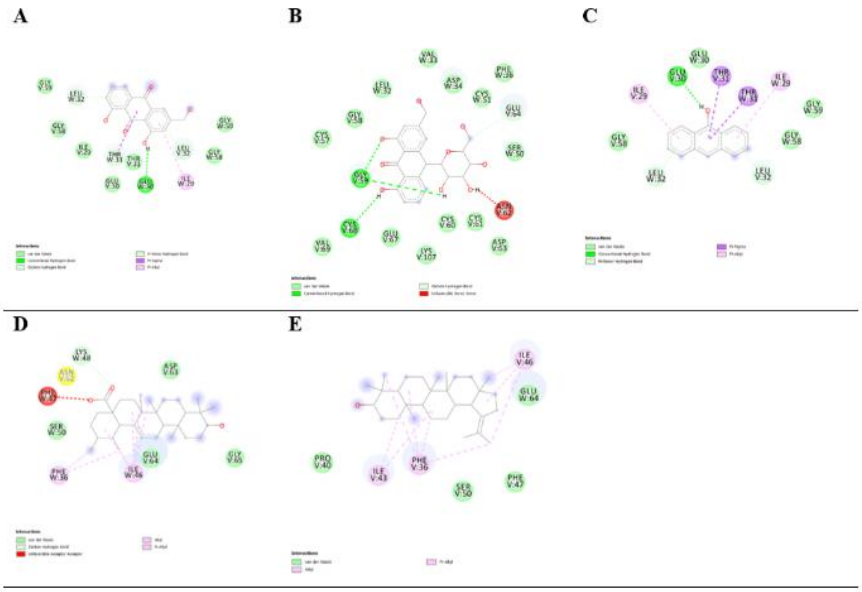

Figure 2. Two-dimensional docking interaction of Aloe vera compounds with VEGF-A.

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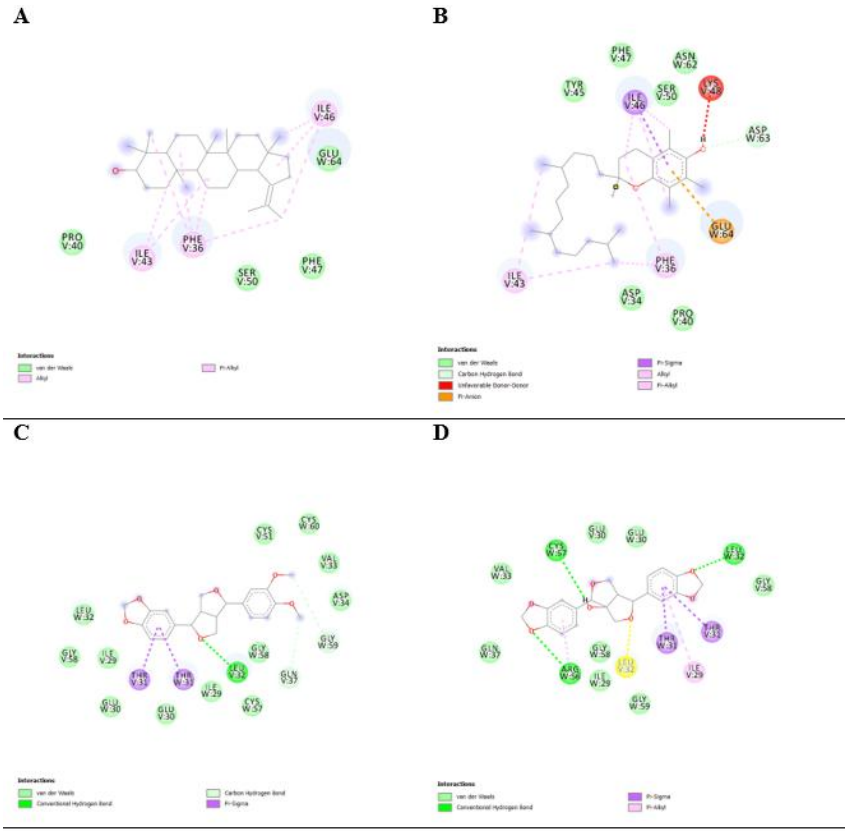

Figure 3. Two-dimensional docking interaction of Andaliman compounds with VEGF-A.

Table 8. Macroscopic wound healing observations

Group	Day 1	Day 4	Day 7	Day 10
Normal				
Placebo				
F1				
F2				
F3				

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Macroscopic wound healing progression was presented descriptively in Tables 8 and 9. Quantitative wound area measurement was not performed because the EB-like lesions appeared as superficial abrasions with irregular wound margins, making diameter or area measurement unreliable. Therefore, the observations focused on visible clinical changes, including erythema, inflammation, granulation tissue formation, epithelialization, and wound closure. The descriptive findings were further supported by histological evaluation and VEGF expression analysis. In vivo experiments confirmed the superior effect of the combination gels, especially F3. Normal and placebo groups exhibited delayed healing, with persistent inflammation and incomplete closure by day 10. F1 improved healing moderately, F2 accelerated closure, and F3 achieved nearly complete epithelialization (Tables 8 and 9). Histological examination (Figure 4) confirmed that F3 produced thick granulation tissue, reduced inflammatory cells, and restored epidermal layers. Moreover, ELISA showed that VEGF expression was significantly increased in F3 ($p < 0.0001$), confirming enhanced angiogenesis (Figure 5).

Table 9. Summary of wound healing progression

Group	Day 1	Day 4	Day 7	Day 10
Normal	Large red wound	Persistent erythema	Limited granulation	Minimal healing
Placebo	Large wound	Inflammation with exudate	Thin granulation	Slow closure
F1	Mild bleeding	Reduced inflammation	Early epithelialization	Partial closure
F2	Wide wound	Faster resolution	Clear epithelialization	Nearly closed
F3	Uniform wound	Rapid reduction	Mature granulation	Complete epithelialization

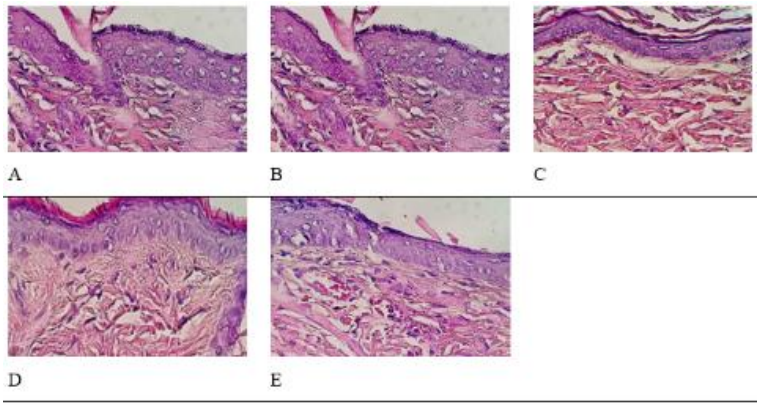

Figure 4. Histological analysis of wound tissue (H&E staining, 400×).

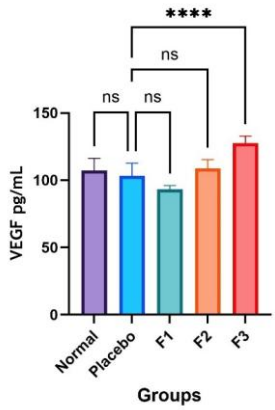

Figure 5. VEGF expression levels after 10 days in the treatment groups. Data are presented as mean ± SD. No significant differences were observed between the normal, placebo, and F1 groups (ns). F2 showed a moderate increase in VEGF expression, while F3 demonstrated a highly significant increase (****$p < 0.0001$) compared with controls, indicating enhanced angiogenesis and tissue regeneration.

The higher VEGF expression observed in the F3 group may indicate a potential synergistic or complementary effect between Aloe vera extract and Andaliman essential oil. This effect may be related to the simultaneous contribution of Aloe vera-derived compounds, such as anthraquinones, sterols, and lupeol, and

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Andaliman-derived constituents, including lignans and antioxidant compounds, in modulating oxidative stress, inflammation, and VEGF-related angiogenic pathways. The in silico docking results also supported the possible interaction of selected compounds with VEGF-A, suggesting their potential role in angiogenesis-mediated wound repair.

However, the synergistic interaction between Aloe vera and Andaliman was not directly confirmed in this study using standard synergy analysis. Therefore, the observed effect should be interpreted as a potential complementary effect rather than definitive pharmacological synergy. Further studies using isobologram analysis or the combination index method are required to quantify the degree of synergy between the individual components.

The phytochemical and GC–MS profiles confirmed the presence of bioactive compounds such as anthraquinones, lignans, sterols, and essential fatty acids that are widely reported to support antioxidant defense, suppress inflammatory pathways, and stimulate angiogenesis. Previous research has shown that anthraquinones from *Aloe vera* accelerate fibroblast proliferation and collagen synthesis [24], while sterols from *Zanthoxylum* species reduce pro-inflammatory cytokine production [25]. The docking analysis in this study strengthens this evidence by revealing that lupeol, anthranol, and paulownin strongly bind to VEGF-A, a central regulator of angiogenesis. These findings align with Banerjee et al. (2020), who demonstrated that triterpenes and lignans enhance VEGF signaling and promote vascularization during tissue repair.

Macroscopic and histological evaluations confirmed that the highest concentration formulation (F3) achieved nearly complete wound closure, with mature granulation tissue and reduced inflammatory infiltration, accompanied by significantly higher VEGF expression (****$p < 0.0001$). These outcomes highlight the role of VEGF in restoring oxygen and nutrient supply to damaged tissues, enabling faster re-epithelialization and remodeling. Comparable results have been reported by Hekmatpou et al. (2019), who showed that *Aloe vera* gel accelerated burn wound healing, and by Manurung et al. (2022), who demonstrated that *Zanthoxylum* extracts improved healing in diabetic ulcers [26,27]. Thus, combining Aloe vera and Andaliman appears to amplify their individual therapeutic properties through complementary mechanisms of action.

Overall, the phytochemical, in silico, and in vivo findings support each other in explaining the wound healing potential of the Aloe vera–Andaliman gel. The phytochemical and GC–MS results indicate the presence of bioactive compounds with antioxidant and anti-inflammatory potential, while molecular docking suggests possible interaction with VEGF-A-related angiogenic pathways. These findings are consistent with the in vivo results, particularly the improved epithelialization, reduced inflammation, and increased VEGF expression observed in the F3 group.

This study has several limitations. The gel stability evaluation was limited to 14 days, and long-term physical, chemical, and microbiological stability has not yet been established. Cytotoxicity and skin irritation tests were also not performed. In addition, only male Wistar rats were used, which may limit the generalizability of the findings across sexes. Histological evaluation was conducted descriptively without semi-quantitative scoring. Therefore, future studies should include long-term stability testing, cytotoxicity assays using skin-related cells, skin irritation evaluation, both male and female animals, and blinded semi-quantitative histological assessment to strengthen preclinical validation before clinical application.

Conclusions

The combination of Aloe vera extract and Andaliman essential oil formulated into a topical gel showed acceptable physicochemical properties and promising wound healing activity in an EB-like superficial abrasion wound model. The F3 formulation demonstrated the most favorable healing profile, supported by improved epithelialization, histological tissue repair, and increased VEGF expression, suggesting a possible role in angiogenesis-mediated wound healing. However, these findings should be considered preliminary. Further studies involving long-term stability testing, cytotoxicity evaluation, skin irritation assessment, and validation in more representative EB models are required before this formulation can be developed for clinical application.

Conflict of Interest

The authors declare that they have no conflicts of interest related to this research.

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Acknowledgment

We would like to express our profound gratitude to Stikes Sehat for all facilities and means during the research. This research received funding from the Directorate of Research and Community Service (Direktorat Penelitian dan Pengabdian kepada Masyarakat, DPPM) Kemdiktisaintek, under grant number 88/SPK/LL1/AL.04.03/PL/2025

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