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ORIGINAL ARTICLE

Comparative Investigation on Wound Healing Potential of Gel Diffusion and Liquid Application of *Adansonia digitata* Active Molecules

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ABSTRACT

Innovation in wound care is increasingly focused on the development of active dressings such as hydrogels. In this study, a poly(acrylic acid)-based hydrogel biomaterial, enriched with a hydroalcoholic extract (80:20) from the stem bark of *Adansonia digitata* (R_1) was synthesized in two formulations: gelled in the presence of KOH (Gel+/R_1) and non-gelled in the absence of KOH (Gel−/R_1). The aim of this work is to compare the wound healing potential by *Adansonia digitata* extract active molecules diffusion of a gelled formulation and the wound healing potential by those active molecules by direct application of a non-gelled formulation on cutaneous injuries induced in Sprague Dawley rats. To attempt this aim, (a) the hydroalcoholic extract (80:20) was characterized using thin-layer chromatography (TLC); (b) a poly(acrylic acid 3000)-based hydrogel incorporating the plant extract (Gel+/R_1) and the non-gelled biomaterial (Gel−/R_1) were formulated; (c) wound healing performance by macroscopic observation of the wounds was evaluated and surface areas (contraction rate) in Sprague Dawley rats were analyzed; (d) hydroxyproline content on Day 12 of wound healing was quantified and finally, (e) microscopic histological analysis on Days 6 and 12 post-induction were conducted. TLC analysis revealed major phytochemical constituents, including flavonoids (catechin), tannins (tannic acid), and phenolic acids (gallic acid). Rats treated with Gel+/R_1 showed significantly higher wound contraction rates compared to the control groups with a wound contraction rate of approximately 97.08 ± 0.6% after 12 days, compared to 92 ± 4.2% in the Gel/KOH-treated group and 93.43 ± 2.3% in the non-gelled biomaterial containing the extract. Hydroxyproline quantification indicated better collagen production for rats treated with Gel+/R_1; histological examination revealed complete re-epithelialization, better organized granulation tissue, fibroblast proliferation, and neovascularization with Gel+/R_1. The Gel+/R_1 hydrogel, with its viscous and translucent texture, provides a favorable diffusion environment for accelerated wound healing and skin regeneration. It represents a promising candidate for the development of bioactive wound dressings. Further studies will investigate its applications in tissue engineering.

Keywords: Wound healing; Gel diffusion; Liquid application; Active molecules; A*dansonia digitata*.

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1.0 INTRODUCTION

The skin possesses a remarkable ability to regenerate and restore its integrity following injury (Liu et al., 2018). It shields internal tissues from mechanical, thermal, and microbial threats (Sadeghi-Aghbash et al., 2023; Ahmed et al., 2024). However, skin wounds caused by physical trauma, burns, surgical procedures, or underlying pathologies remain a major clinical challenge (Rodrigues et al., 2018; Yu et al., 2022). Effective wound care is crucial aiming to relieve pain, prevent infection, and accelerate tissue regeneration (Xu et al., 2023). The global wound care market reflects this need, with its value expected to climb from $12 billion in 2020 to $18.7 billion by 2027 (Albahri et al., 2023). Among the medical devices designed to address this challenge, "active" dressings have become essential. They must meet several criteria, including maintaining a moist environment, managing exudates, and ensuring biocompatibility (Xiang et al., 2020). Hydrogels have emerged as a leading material for wound dressings. These three-dimensional networks of hydrophilic polymers (Chang et al., 2010), can offer excellent capacity for incorporating and releasing bioactive agents (Francisko et al., 2018), while mimicking the extracellular matrix (ECM) due to their gel-like texture. This is ideal for promoting cell adhesion, migration, and proliferation, as well as modulating inflammatory mediators (Patrulea et al., 2015; Nacu et al., 2023). Various hydrogels are currently used for wound healing, including Geliperm, Curasol, Tegagel, Biolex, Carrasyn, NuGel, and Exu-Dry Dressing Kamoun et al. (2017) these formulations are derived from either natural or synthetic polymers (Luca et al., 2023). Poly(acrylic acid), commonly known as Carbopol, is a widely used synthetic polymer due to its excellent swelling capacity, bioadhesion, biocompatibility, and biodegradability (Khanlari et al., 2015; Tamburic et al., 1995). Several studies have demonstrated the efficacy of Carbopol-based hydrogels, particularly when combined with natural bioactive substances such as *Aloe vera* extract (Meza-Valle et al., 2021; Saberian et al., 2021) or *Basella alba* extract (Haneefa et al., 2012). Notably, about 30% of herbal medicines have shown promising wound healing properties (Xu et al., 2023).

In a previous work, we evaluated the healing potential of a Carbopol-based gel formulation, containing *Adansonia digitata* stem bark extract in Wistar rats (Gaffo et al., 2022). *Adansonia digitata* has been subjected to various studies for its pharmacological activities such as antimicrobial, anti-inflammatory, antioxidant, and properties; Its trunk bark particularly has been cited in various ethnopharmacological surveys for its wound healing properties Gaffo et al. (2022). Then, we have applied mixtures of Carbopol 974P NF solution containing 2.5% and 5% of 50:50 water/ethanol extract.

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In the present study we propose two new formulations based on poly(acrylic acid 3000) hydrogel, incorporating 2.5% of a hydroalcoholic (80:20) extract of *Adansonia digitata* (Gaffo et al., 2026). A gel form was obtained with KOH addition and a non-gelled liquid form without KOH addition. The aim of this study is then to compare *Adansonia digitata* extract active molecules wound healing efficacity in the gel formulation *versus* in the non-gelled liquid formulation.

2.0 MATERIALS AND METHODS

2.1 Plant material collection and identification

The stem bark of *Adansonia digitata* was harvested on April 13, 2022, in Lomé, Togo (geographic coordinates: 6°11'35.7"N, 1°10'04.2"E). A plant specimen was identified with the help of botanists of Botany and Plant Ecology Laboratory, University of Lomé (Togo).

2.2 Plant material preparation

After collection, bark samples were cut into small pieces and thoroughly rinsed with tap water to remove impurities. The pieces were then air-dried for seven days in a climate-controlled room at 27 ± 2°C. Once dried, the bark fragments were broken for phytochemical extraction.

2.3 Hydroethanolic extraction of *Adansonia digitata* bark

A total of 100 g of the prepared stem bark fragments was macerated in 1 L of a hydroethanolic solution (80:20, v/v water/ethanol). The maceration was performed at ambient temperature (27 ± 2°C) for 72 hours with intermittent stirring to facilitate the extraction of secondary metabolites. Following maceration, the mixture was filtered using Whatman No. 3 filter paper. The resulting filtrate was then concentrated under reduced pressure using a rotary evaporator (model A.R.) at 45 °C to obtain a viscous crude extract by removing the solvents.

2.4 Phytochemical characterization by thin layer chromatography (TLC)

The concentrated extract was subjected to qualitative analysis using thin layer chromatography (TLC), following the method by Akpalo et al. (2022). Silica gel 60 F254 plates (0.25 mm thickness) were used for the analysis. Elution was performed in an ascending phase with a mobile phase consisting of ethyl acetate and ethanol in a 1:1 (v/v) ratio, which was optimized to ensure effective separation of compounds based on polarity. After migration, the plates were observed sequentially under UV light at 254 nm (fluorescence quenching) and 366 nm (fluorescent compound detection). The plates were then sprayed with specific chemical reagents to reveal the presence of particular phytochemical groups: vanillin-sulfuric acid for terpenoids and steroids; aluminum chloride for flavonoids; Liebermann-Burchardt reagent for steroids and triterpenes; ferric chloride for phenolic compounds and tannins; finally, ammonia, sodium or potassium hydroxide which are basic developers. Depending on the reagent used, plates were either heated or exposed to UV light to visualize the compounds. The retardation factor (Rf) for each compound was then calculated using the following formula:

$$Rf = \frac{d}{D} \quad (1)$$

Where;
d: Deposition distance to spot.
D: Deposition distance to front line.

2.5 Synthesis of the hydrogel biomaterial containing *Adansonia digitata* extract

The hydrogel biomaterial was synthesized to incorporate the *A. digitata* extract. Two distinct formulations, a gel form and a non-gelled liquid form, were prepared to evaluate the effect of gelation on wound healing.

2.5.1 Preparation of the gel form with KOH

The hydrogel biomaterial Gel/KOH was synthesized by dispersing 2.5% of poly(acrylic acid) polymer (PAA3000) in distilled water. The mixture was homogenized using a magnetic stirrer for one hour at 250 rpm and at a constant temperature of 50 °C. For Gel+/R_1, resinous crude extract of *A.digitata* was incorporated into the reaction mixture at a final concentration of 2.5% means 2,5g/100mL. It is well noted that resinous crude extract of *A.digitata* was obtained using a 80:20 hydroethanolic ratio, the best ratio for the formulation among 20:80, 50:50 and 80:20. The entire preparation was stirred at 250 rpm for an additional hour always at 50 °C. Gelation was induced by the gradual addition of 16 mL of a 1 M potassium hydroxide (KOH) solution under stirring until a homogeneous, viscous gel was obtained. For Gel/KOH, the formulation without extract, only 1.3 mL of a 1 M potassium hydroxide (KOH) solution were needed to induced gelation. Gelation was indicated by the automatically stop of stirring.

2.5.2 Preparation of the liquid form without KOH

The preparation procedure for the non-gelled liquid form (Gel-/R_1) was identical to that of the gelled hydrogel, with the key exception that the gelation step after resinous crude extract of *A. digitata* incorporation, was omitted. The pH was measured for each formulation using a digital pH meter. The preparation of all formulations was repeated three times and then their pH recorded in triplicate. The reported values correspond to the average of the three measurements.

2.6 Experimental animals and housing conditions

Concerning experimental animals, the study received the ethical approbation (n°010/2021/BC-BPA/FDS-UL) from Animal Physiology Department of Sciences Faculty. For animal material, male and female Sprague Dawley rats, aging from 6 to 8 weeks old and weighing between 80 and 100 g, were used in this study. The animals were bred at the Physiology/Pharmacology Laboratory Animal Facility, Faculty of Sciences. Prior to the experiment, the animals underwent a 10-day acclimatization period. They were housed in wire mesh cages under standard laboratory conditions: temperature of 24 ± 2°C, a 12-hour light/dark cycle, and a diet of standard rat pellets and tap water provided *ad libitum*.

2.7 Ethical approval

Concerning in vivo experimentations, the study received the ethical approbation (*N°010/2021/BC-BPA/FDS-UL*) from Animal Physiology Department of Sciences Faculty.

2.7.1 Induction of cutaneous wounds by excision

Male and female Wistar rats (commonly used in all pharmacological tests), weighing between 80 and 100 grams, were used. These animals were raised in the animal facility of the Physiology/Pharmacology Laboratory at the Faculty of Sciences of the University of Lomé. The rats had free access to food and water and were kept at an ambient temperature of approximately 25°C and on a 12-hour light/dark cycle. Circular, full-thickness excision wounds with a diameter of 1.5 cm were created on the shaved dorsal region of the rats. The procedure was performed under ether anesthesia using an open mask technique, as per the protocol of Gilles et al. (2011). The animals' backs were then shaved using an electric clipper and cleaned with 70% alcohol. Using surgical scissors, the area marked by a 1.5 cm-diameter ink pad was excised to create a circular surface. To maintain aseptic conditions, the procedure was conducted with strict adherence to sterility. Topical treatments (Gel/KOH, Gel+/R1, Gel-/R1 and L-Mesitran) were applied once daily for the first three days following wound induction. Wounds were then left uncovered, and animals were housed individually (to avoid dorsal licking among animals) in separate cages for twelve (12) days. The total experiment duration was twenty-two (22) days.

2.8 Experimental design

Rats were randomly divided into four groups of eight individuals each (4 males and 4 females as healing is not dependent of sex):

Group I *(Gel/KOH):* Treated by topical application (2 mg spread) of poly(acrylic acid)-based hydrogel without extract.
Group II *(Gel+/R_1):* Treated by topical application (2 mg spread) of the gel form of poly(acrylic acid)-based hydrogel with *A. digitata* extract.
Group III (Gel–/R_1): Treated by topical application (2 mL spread) of the non-gelled liquid form of poly(acrylic acid)-based hydrogel with *A. digitata* extract.
Group IV (Reference): Treated by topical application of L-Mesitran, a commercial honey wound healing hydrogel.

2.9 Monitoring of wound surface area

Wound healing progression was evaluated by daily measurement of the wound area using digital photography and the ImageJ software (Amégbor et al., 2012). The same experimental researcher made all the photographs always at the same distance of 27 cm with a Samsung Galaxy A5 smartphone, when the animals are still anesthetized. The percentage of

wound contraction (Tx) was then calculated for each day using the following formula:

$$\text{Percentage of wound contraction (Tx)} = \frac{Sj0 - Sjx}{Sj0} \text{ X } 100 \quad (2)$$

Where;
Tx: Percentage of wound contraction.
Sjx: Wound surface area on Day x.
Sj0: Wound surface area on initial Day.

2.10 Histological analysis

On the sixth Day, two rats from each group were sacrificed by overdose of ether anesthesia (in complete safety) and biopsies were used for histological examinations. On the twelfth Day, two rats from each group were again sacrificed always by overdosage of ether anesthesia (in complete safety) for histological tests. For these different tests, an excision of about 1cm in diameter from the scar was taken. Tissue samples from the wound area were fixed in 10% buffered formalin. Following paraffin embedding, thin sections were cut using a microtome and stained with hematoxylin and eosin (H&E). The slides were examined under a light microscope at various magnifications by a pathologist (Olympus BX 51 light microscope; 10 µm for scale bar). The following qualitative parameters were assessed to evaluate the healing process: granulation tissue formation, re-epithelialization, neovascularization, inflammatory cell infiltration, and fibroblast proliferation.

2.11 Hydroxyproline assay

On Day 12, all remaining animals were anesthetized by ether anesthetic overdose (in complete safety). Fragments of scar tissue were collected to determine hydroxyproline content, a reliable indicator of collagen synthesis. The samples were hydrolyzed in hot 6N HCl and analyzed using the colorimetric method by Darré et al. (2014).

2.12 Statistical analysis

All data were expressed as mean ± standard error of the mean (Mean ± SEM) giving the true population average. Group comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test using GraphPad Prism software (version 8.4.2). Differences were considered statistically significant at $p < 0.05$.

3.0 RESULTS

3.1 Thin layer chromatography (TLC)

The TLC analysis of the *Adansonia digitata* extract revealed the presence of various groups of phytochemical compounds. The spots observed on the chromatographic plate were predominantly coloured in orange, light blue, and dark blue (Plate 1), indicating the diversity of metabolites presents. With or without specific detection reagents, these colors corresponded to classes of compounds such as flavonoids, coumarins, tannins, methylated flavones, terpenes, and polyhydroxyflavones. The addition of detection reagents enhanced the identification of certain reference molecules or their analogs in the extract, based on the color of the spots and their retention factor (Rf). The key findings under visible light with the detection reagents (Plate 1) were as follows: with the Vanillin sulfuric acid reagent, the orange spot at Rf = 0.77 corresponds to catechin (flavonoid) then the orange spot in the extract at Rf = 0.77 should corresponds to flavonoids; however, it also revealed terpenes since matching with any reference molecule, belongs to terpenes family. With ferric chloride ($FeCl_3$), the blue spots at Rf = 0 corresponds to gallic acid (phenolic acid) and tannic acid (tannin); then the blue spot with Rf value = 0 in the extract, exhibits the presence of tannin in this one. With the $AlCl_3$ reagent, the yelow spot at Rf = 0 corresponds to flavonoid since matching with any reference molecule. With the Liebermann-Burchardt reagent, the orange spot at Rf = 0.72 corresponds to catechin (flavonoid) and is the same in the extract. Finally, with any detection reagent, the orange spot at Rf = 0 corresponds to flavonoids under visible light and and the blue spot at Rf = 0.77 under UV 366nm corresponds to flavonoids, terpenes, sterols, since matching with any reference molecule. The full set of revealed phytochemical compounds and their characteristics (color, Rf, chemical class) are summarized in Table 1.

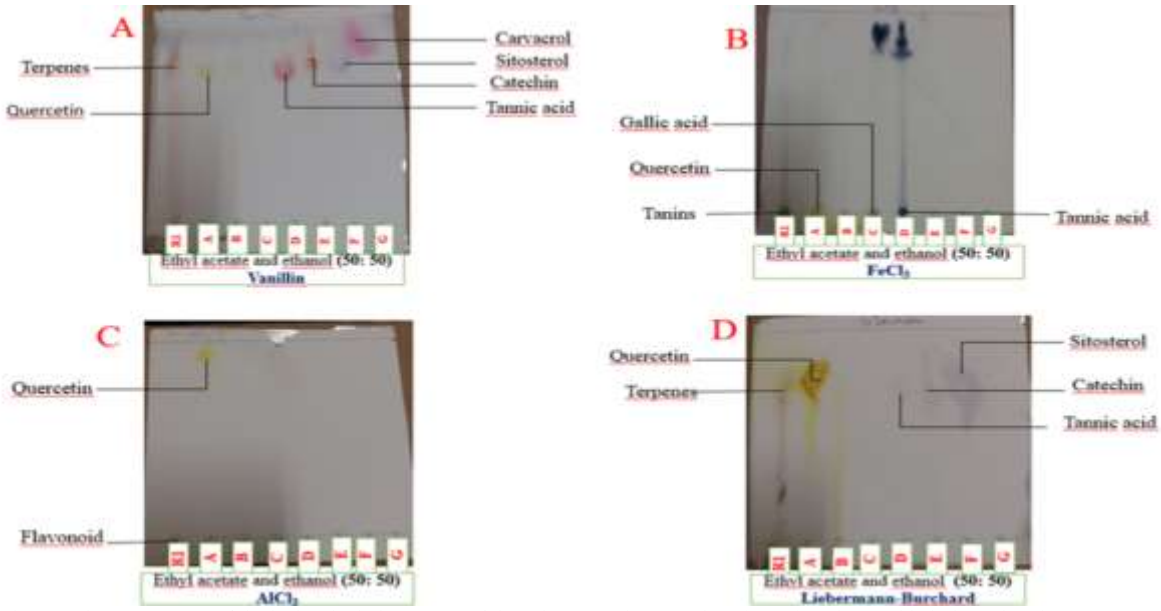


*Plate **1**. Chromatograms of the hydroalcoholic extract of the Adansonia digitata plant (80:20) under Visible light and in the presence of developers such as: $FeCl_3$ (A); sulfuric vanillin (B); $AlCl_3$ (C); Liberman Buchard (D). Symbols: R_1 = extract (80:20); A= Quercetin B= Rutin C= Gallic acid D= Tannic acid E= Catechin F= Sitosterol G= Carvacrol.*



PAA-3000 hydrogel in the presence of KOH without extract (Gel/KOH)

PAA-3000 in gel form in the presence of extract with KOH (Gel^{+}/R_1)

PAA-3000 in non-gelled liquid form in the presence of extract without KOH (Gel^{-}/R_1)

*Plate **2**. Photographs of macroscopic aspects of the various hydrogel biomaterials obtained.*

Table **1**. Phytochemical compounds revealed by CCM analysis

Extracts				**R_1 (80/20)**	
Revelators	Criteria	Rf	Spot color	Possible compound group	Identified reference molecules
Sans révélateurs	Visible light	0	Orange	Flavonoids	
	UV366	0.77	Blue	Flavonoids ; Terpenes, Sterol	
Vanillin	Visible light	0.77	Orange	Terpenes	Catechine
	UV366	0.77	Blue	Terpenes ; Polyhydroxyflavone	
$FeCl_3$	Visible light	0	Blue	Tanins	Gallic acid, Tannic acid
	UV366	-	-	-	-
$AlCl_3$	Visible light	0	yelow	Flavonoid	-
	UV366	0,77	Blue	Coumarines ; Flavones methyled	-
Liberman Buchardt	Visible light	0.72	Orange	Sterol; Terpenes	Catechin
	UV366	0.77	Blue	Terpenes	-
Anysaldehyde	Visible light	0	Orange	Terpenes	-
	UV366	0.77	Blue	NI	-
KOH	Visible light	-	-	--	-
	UV366	0.77	Blue	Coumarines ; Methylated flavones	-
NH_3	Visible light	-	Pale yellow streak Blue	Coumarins umbelliferone	-
	UV366	0.77		Coumarins; Tanins; Flavonoids	-
	Visible light	-	Pale yellow streak	Coumarins umbelliferone	

3.2 Forms of the hydrogel biomaterial containing *Adansonia digitata* extract

The hydrogel was synthesized under two conditions: in the presence or absence of KOH. Plate 2 presents the different forms obtained under the respective experimental conditions. In the presence of KOH, the PAA 3000 biomaterial appeared in a gelled form, with a soft, viscous, and transparent texture (Gel/KOH). In the absence of KOH, the mixture remained in a liquid and foamy state without forming a structured gel. When *Adansonia digitata* extract was incorporated into the system, the gel in the presence of KOH ($Gel+/R_1$) adopted an orange color, with no visible change in texture compared to the control gel (Gel/KOH). The system without KOH but with the extract ($Gel-/R_1$) became liquid and foamy, also displaying the orange tint. Illustrative images of these different conditions are grouped in Plate 2. Table 2, presents control, L-Mesitran and the extract two formulations' pH.

Table **2**: pH of obtained materials obtained

Type of materials	**pH**
Gel/KOH	3.5
$Gel+/R_1$	6.36
$Gel-/R_1$	3.4
L mesitran	Honey's acidic pH 3.2 to 4.5

3.3 Macroscopic observations of the healing and wound surface variation

Macroscopic observation of the wounds and analysis of their surface areas showed a progressive reduction over time. A considerable decrease was already visible by Day 3 in the groups treated with the $Gel+/R_1$ biomaterial, compared to both the control group and the group treated with the non-gelled biomaterial $Gel-/R_1$ (Plate 3). The results of the study showed that rats treated with $Gel+/R_1$ showed significantly higher wound contraction rates compared to the control groups with a wound contraction rate of approximately 97.08 ± 0.6% after 12 days, compared to 92 ± 4.2% in the Gel/KOH-treated group and 93.43 ± 2.3% in the non-gelled biomaterial containing the extract (Figures 1 and 2).

In rats from the L-Mesitran group, wound contraction rates were as follows: 36.084 ± 3.981% on Day 3 (D3), 58.314 ± 3.98% on Day 6 (D6),80.14 ± 1.50% and on Day 9 (D9); in rats from Gel/KOH treated group, wound contraction rates were as follows (25.084 ± 4.35% on D3; 57.16 ± 1.87% on D6; 89.47 ± 3.1% on D9) (Figures 1 and 2). Closure rates of each formulation before reached a plateau was 22.7% per day for the first 3 days for $Gel+/R_1$ then at 2.8% per day until day 12. 17.5% per day also for the first 3 days for $Gel-/R_1$ then at 4.3% per day until Day 12, 9.4% per day until day 9 for the control group and 10.1% per day until Day 9 for L-Mesitran. Indeed, by Day 12, $Gel+/R_1$ group achieved a wound closure rate of 97.08 ± 0.6%, a significant improvement compared to $Gel-/R_1$ (93.43 ± 2.3%) and the control group (92 ± 4.2%) without extract.

3.4 Hydroxyproline content in scar tissue fragments

In our study, a significant increase in hydroxyproline content was observed in scar tissue fragments from rats treated with the $Gel+/R_1$ biomaterial (0.445 ± 0.012 µg/mg). Compared to the negative control group Gel/KOH (0.271 ± 0.021µg/mg), this increase was highly significant ($p < 0.005$). It was also significant when compared to the $Gel-/R_1$ group (0.303 ± 0.013 µg/mg; $p < 0.02$) and to the group treated with L-Mesitran (0.264 ± 0.028 µg/mg; $p < 0.002$) (Figure 3). These results suggest that treatment with

Gel+/R_1, which combines the hydrogel and the *Adansonia digitata* extract, significantly promotes collagen production at the wound site, thereby indicating an improved healing process through enhanced extracellular matrix stimulation.

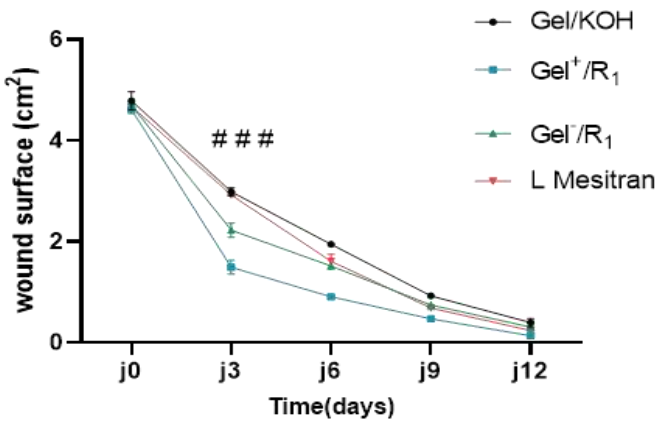


*Figure **1**. Changes in wound surface area over time. Values are expressed as means ± SEM, n = 8 (the experiment was repeated twice). Area units: cm². # # # #p < 0.0001: Gel+/KR_1 vs. Gel/KOH vs. Gel-/R_1 vs. L mesitran from Day 3 to Day 9 (this refers to the comparison between the Gel+/R_1 group and the other groups on Day 3).*

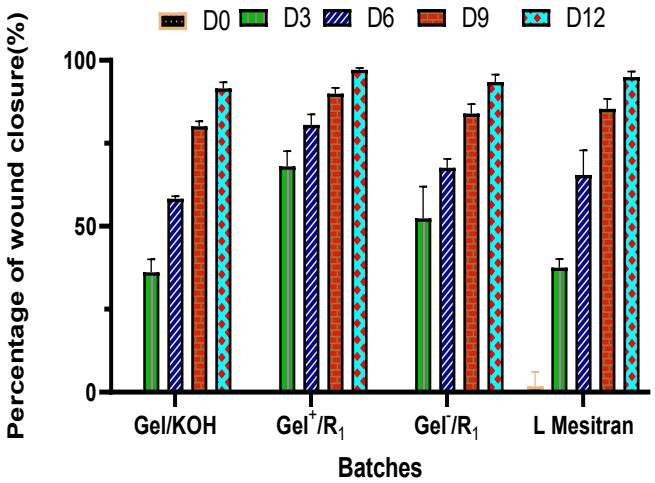


*Figure **2**. Closing rate of wound surfaces over time: progression of wound closure expressed as a percentage. A statistical comparison of these groups yields: **** p < 0.0001: Gel+/R_1 vs. Gel/KOH vs. Gel-/R_1 vs. L Mesitran from Day 3 to Day 9 (this refers to the comparison between the Gel+/R_1 group and the other groups on Day 3). ##p < 0.0096: Gel+/R_1 vs. Gel-/R_1 on Day 9 (this refers to the comparison between the Gel+/R_1 group and Gel-/R_1 groups on Day 9). *p < 0.0054: Gel+/R_1 vs. Gel/KOH on Day 12 (this refers to the comparison between the Gel+/R_1 group and Gel/KOH groups on Day 9).*

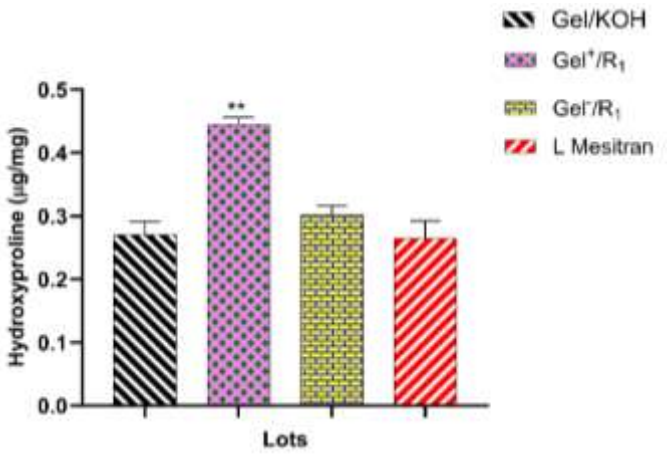


*Figure **3**: Hydroxyproline content of different batches. Values are expressed as Means ± MSE, n = 6. Units: µg/mg dry tissue. **p< 0.0057: Gel+/R_1 vs Gel/KOH (this refers to the comparison between the Gel+/R_1 group and Gel/KOH groups). *p< 0.0220: Gel+/R_1 vs Gel-/R_1 (this refers to the comparison between the Gel+/R_1 group and Gel-/R_1 groups). **p< 0.0220: Gel+/R_1 vs L Mesitran (this refers to the comparison between the Gel+/R_1 group and Gel-/R_1 groups).*

3.5 Wound healing histological effect of gel diffusion or non-gelled liquid application of *Adansonia digitata* active molecules

Newly synthesized skin tissues were analyzed through histopathological tests using different staining techniques (Hematoxylin & Eosin and Masson's Trichrome). Microscopic histological examination of the wounds revealed the presence of fibroblasts and early epithelialization in rats treated with Gel+/R_1 (Plate 4G and H), compared to the Gel/KOH group (Plate 4A and B), which still exhibited ulcerations. At the twelfth Day, rats treated with Gel+/R_1 showed clear epithelialization and a higher density of developed fibroblasts, in contrast to the Gel/KOH group, which displayed poor fibroblast proliferation. This proliferation was also less pronounced in the groups treated with Gel−/R_1 and L-Mesitran. Rats in Group I that received topical application of the Gel/KOH (negative control) demonstrated significantly lower cutaneous tissue regeneration. The viscous and translucent nature of Gel+/R_1 allows it to form a protective film over the wound and facilitates a gradual release of the active ingredients; contrary, the Gel-/R_1 group, although containing the extract, performed less effectively as the formulation not adhere and stay on the wound.–Moreover, at the cellular level, tissue samples from this group showed an increased presence of inflammatory cells key mediators of wound healing scattered across the wound area, along with a decrease in granular cells (immature skin). These findings indicate reduced skin tissue regeneration and, consequently, delayed wound recovery (Plate 4A and B).

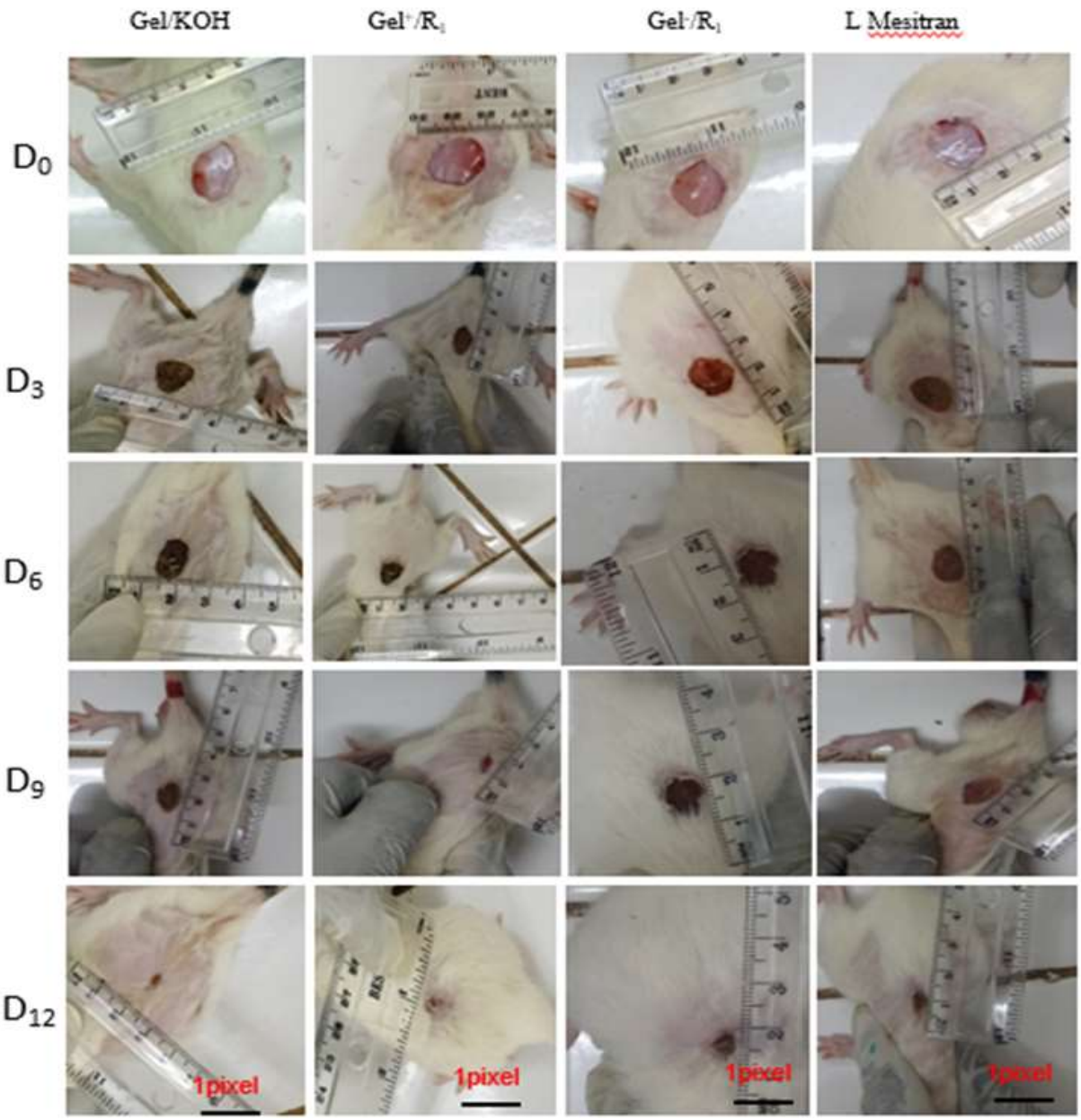
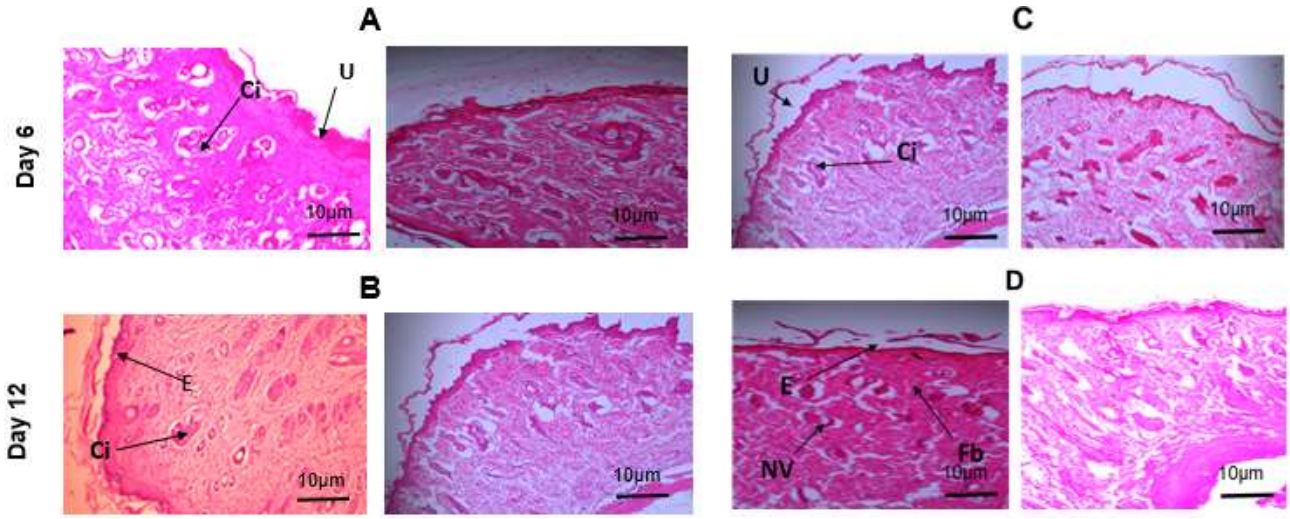


Plate **3**. Images of wound surfaces over time. (Scale bar is 1pixel for 1 cm).



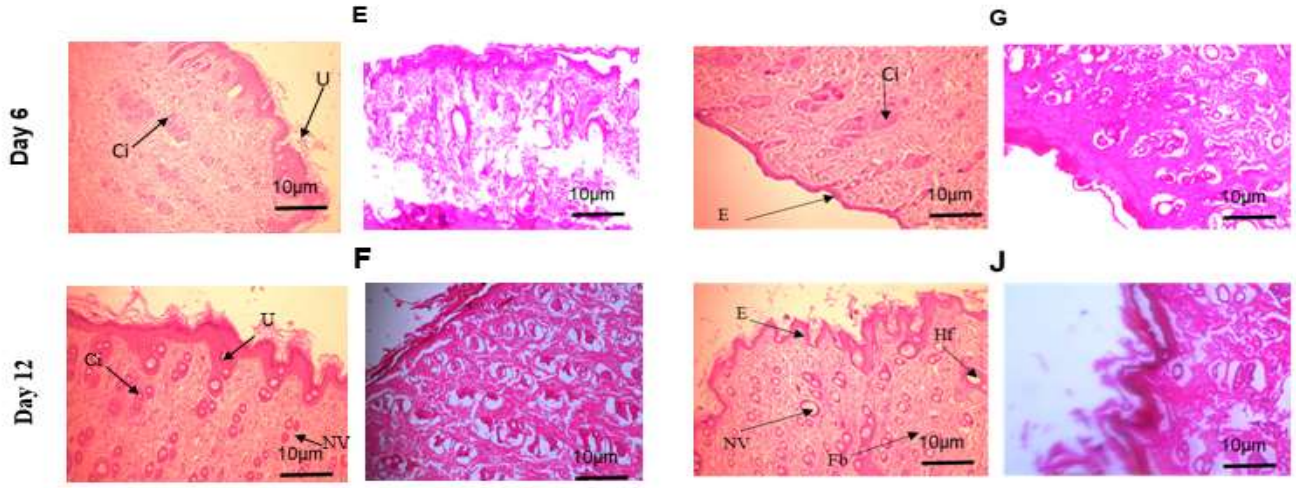


Plate 4: Histological observation of wounds on Day 6 and Day 12. A = Gel/KOH; B= Gel/KOH l; C= Gel-/R1; D= Gel-/R1 ; E=L Mesitran; F=L Mesitran ; G= Gel+/R1; H= Gel+/KR1 Nv = Neovascularization; Hf = Hair follicle; Fb = Fibroblast; U= Ulceration; E = Squamous epithelium; Ci=inflammators cells. Hematoxylineosin (H&E) staining. Olympus BX 51 light microscope. Scale bar is 10 µm.

4.0 DISCUSSION

First, thin-layer chromatography revealed the presence of flavonoids-like molecules structurally similar to catechin, phenolic acids-like molecules structurally similar to gallic acid, and tannins-like molecules structurally similar to tannic acid, all known for their anti-inflammatory, antioxidant, astringent, and antimicrobial properties (Kumar et al., 2015). The flavonoid and triterpenoid present in *Hyptis suaveolens* leaves have been demonstrated to play a key role in wound healing properties (Mokwenye et al., 2023) In 2018, Zheng et al. have showed that tannic acid loaded in an injectable gelatin–gellan based hydrogel system significantly accelerated wound closure promoting highly aligned collagen fiber deposition (Zheng et al., 2018). Several studies have indicated that phytochemicals such as flavonoids, tannins, and phenols possess high antioxidant capabilities and then scavenge free radicals and chelate metal ions, lowering oxidative stress and associated damage (Anigboro et Otabor, 2024). Traditional medicine has long used plant parts to create topical preparations that promote wound healing (Xu et al., 2003).-Then, poly(acrylic acid) is an ideal polymer for tissue engineering applications due to its excellent gelling properties and its beneficial effect on stasis zones (Arabpour et al., 2024). More, it possesses muco-adhesive properties and its bio-adhesive potential has been investigated (Sarfaz et al., 2022). In fact, majority of conventional hydrogels involved in dermatological patches are composed of poly(acrylic acid) and its salts (Nishikawa et al., 2008). We compared two formulations based on poly(acrylic acid), hydrogel (Gel+/R_1) to a non-gelled liquid (Gel-/R_1) and a control group (Gel/KOH) to understand the effect of gelling on the plant extract's action. The pH 6.36 of the Gel+/R_1 close to neutral, made it optimal by matching the safe pH range of skin (4 – 6.5) (Yu et al.; 2022, Hendi et al.; 2020). From a macroscopic perspective, rats treated with Gel+/R_1 showed faster wound healing from the very first days, with a visible reduction in the lesion diameter. These differences are explained by the ability of the Gel+/R_1 hydrogel to maintain a moist environment; indeed, hydrogels are applied as scaffolds in tissue engineering as they facilitate the wound healing process thanks to a moist and occlusive environment, they give (Chummun et al., 2021). The diffusion of water and the gel's hydrating properties create a milieu conducive to tissue regeneration. Hydrogels are highly porous systems which allow filtration, migration and proliferation of cells just as blood vessel ingrowth. Unlike a dry environment, a moist wound allows epithelial keratinocytes to migrate more efficiently to re-epithelialize the surface (Nuutila et al., 2021; Chummun et al., 2021). Another evidence of the effectiveness of Gel+/R_1 is the advantage of hydrogels to encapsulate bioactive agents which afterwards undergo sustained release (Chummun et al., 2021). Peng et al. (2012) and Chen et al. (2013) have demonstrated that Astragaloside IV loaded in alginate-gelatin hydrogels or in solid lipid nanoparticles (SLNs) embedded in carbomer hydrogel was respectively more effective in re-epithelization of acute wounds and regenerated skin with mainly type I collagen than the Astragoloside IV solution (Peng et al., 2012; Chen et al., 2013). About hydroxyproline content, the effectiveness of Gel+/R_1 was confirmed by a biochemical analysis of hydroxyproline, an indirect marker of collagen synthesis. The healed tissues from the Gel+/R_1 group showed a significant increase in hydroxyproline compared to the Gel-/R_1 group. This increase, at 0.445 ± 0.012 µg/mL/mg, indicates a greater stimulation of collagen deposition. The porous and biomimetic structure of the hydrogel promotes this effect by maintaining hydration and stimulating fibroblasts, the collagen-producing cells (Nuutila et al., 2021). Although we were unable to identify the specific types of collagens produced, their increase is directly correlated with better strength and integrity of the scar tissue (Iyyam et al., 2010). Histological examination also corroborated these observations. As early as Day 12, rats treated with Gel+/R_1 showed complete epithelialization, an increased density of fibroblasts, and better vascularization. Unlike the control group and Gel-/R_1, no ulceration or inflammatory infiltration was observed, a sign of a healthy healing progression. Our results demonstrate that the formulation of a poly(acrylic acid)-based hydrogel enriched with *A. digitata* extract constitutes a promising biomaterial for wound healing. Its ability to create a moist environment for bioactive compounds, stimulating cell proliferation and collagen synthesis, makes it a superior approach to non-gelled extracts.

Conclusion

In this study, our objective was to compare the wound healing efficacy of *Adansonia digitata* active molecules in the gelled and non-gelled (liquid) formulations. The gelled biomaterial (Gel+/R_1) proved to be particularly effective. It promoted rapid healing, significant wound contraction, and increased collagen synthesis compared to the non-gelled (liquid) formulation (Gel-/R_1). This effectiveness is due to the sustaining release properties of hydrogels and the bioactive compounds in the plant extract. Histological analyses confirmed these observations, showing complete re-epithelialization and better dermal regeneration in the hydrogel-treated group.All this suggests that our biomaterial gel properties are crucial and contributes to maintain conditions favorable for optimal healing by hydrogel. These results position this biomaterial as a promising candidate for clinical applications.

Future work will be necessary to more fully characterize this biomaterial according to tissue engineering standards, paving the way for its development for medical use.

Conflict of interests

The authors have not declared any conflict of interests.

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Authors' contributions

AEA conceived the research idea, analyzed the data and wrote the draft. ARG made extractions, wound excisions, biomaterial applications, analyzed the data and wrote the draft. HS provided technical assistance and made valuable comments on the manuscript. KEG gave valuable scientific critical advises.

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