RESEARCH ARTICLE

Phytochemical Validation and Wound Healing Efficacy of a Polyherbal Gel using HPLC and Zebrafish Models

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ABSTRACT

Background: Wound healing is a multifaceted physiological process involving inflammation, tissue regeneration and remodeling. Silver sulfadiazine remains a conventional topical treatment; its clinical use is often limited by delayed epithelialization and cytotoxicity. Medicinal plants, rich in diverse bioa ctive compounds, are recognized as safer alternatives for wound management. Hence, this study aimed to formulate a standardized polyherbal gel using *Trigonella foenum-graecum, Lawsonia inermis, Basella alba, Peristrophe paniculata and Portulaca oleracea* and to evaluate its phytochemical composition and wound-healing efficacy.

Methods: A Carbopol-based gel incorporating hydroalcoholic extracts of the selected plants was developed. High-performance liquid chromatography (HPLC) was employed to identify and quantify key phytoconstituents, protocatechuic acid, syringic acid, kaempferol, apigenin and diosmetin. *In vivo* evaluation was conducted using zebrafish (Danio rerio) wound models, including superficial mechanical injury and tail fin transection, with wound closure assessed for seven days. Histopathological analyses were also performed.

Results: HPLC analysis confirmed the stability and quantifiable presence of bioactive phytochemicals, with excellent linearity ($R^2 > 0.999$). The polyherbal gel demonstrated significant wound-healing activity, with wound closure rates of 88.7% ± 2.7 in mechanical injury and 94.5% ± 2.1 in tail fin transection by Day 7, outperforming untreated controls and exhibiting superior efficacy to 1% silver sulfadiazine. Histopathological assessment revealed organized re-epithelialization, restored fin structure and reduced inflammation in gel-treated groups.

Conclusion: The formulated polyherbal gel offers a safe, biocompatible and efficacious topical therapy for wound healing. Its synergistic phytoconstituents contribute to enhanced regeneration, supporting its potential for further preclinical and clinical translation in wound care.

Keywords: Polyherbal gel, wound healing, zebrafish model, HPLC, flavonoids, phenolic acids

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INTRODUCTION

Wound healing is a vital biological process that involves a complex interplay of cellular and molecular mechanisms. The healing process of the wound is generally divided into overlapping stages: hemostasis, inflammation, proliferation and remodeling. All of these work together to repair and restore the functions of injured tissues. Interruption to these stages can lead to slow or impaired healing, resulting in chronic wounds. Such chronic wounds represent a major global health concern, affecting millions of people each year.

These lead to a reduction in quality of life and result in a significant economic burden on healthcare systems[1,2]. Despite progress in wound management methods worldwide, the ongoing need for effective, affordable and biocompatible treatments remains a significant challenge to modern medicine. The complexity of the wound microenvironment, shaped by various internal and external factors that can delay healing, necessitates innovative approaches to treatment development[3].

Conventional medical practices have historically depended on plant-derived management for wound care and skin conditions. For example, polyherbal formulations, which utilize various medicinal plants, have evolved due to their potential synergistic benefits. Unlike single-herb preparations, polyherbal medications are thought to provide a broad therapeutic spectrum, addressing the various stages of wound healing, including oxidative stress, inflammation, microbial invasion and tissue regeneration[2].

This ethnopharmacological rationale aligns with the modern biomedical insights, positioning polyherbal strategies as promising for the development of topical wound care agents. Based on this concept, the current study focuses on the formulation and evaluation of a novel polyherbal gel comprising five medicinal plants: *Trigonella foenum-graecum (fenugreek), Lawsonia inermis (henna), Basella alba (Malabar spinach), Peristrophe paniculata and Portulaca oleracea (purslane)*. Each plant was selected based on its significance in ethnomedicine and the documented pharmacological characteristics. *T. foenum-*

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graecum is recognized for its content of flavonoids and trigonelline, which offers antioxidant and anti-inflammatory effects; *L. inermis* is abundant in lawsone and related naphthoquinones, providing strong antimicrobial properties; *B. alba* contains mucilage and phenolic compounds that support hydration and also aids in healing epithelial tissues; *P. paniculata* has been traditionally used for its antimicrobial and anti-inflammatory properties; and *P. oleracea* is high in omega-3 fatty acids, vitamin C and flavonoids, delivering both antioxidant and anti-inflammatory effects. Altogether, these bioactive-rich plants are contemplated to provide effects that accelerate wound closure and enhance tissue repair.

For these herbal formulations to gain clinical acceptance, scientific validation and standardization are ideally essential. The basic preparation of a polyherbal gel is insufficient without examining the presence and stability of key active compounds. High-Performance Liquid Chromatography (HPLC) is a widely used technique for profiling phytochemicals in complex formulations, providing reliable results and ensuring quality control. In this study, HPLC was used to identify and quantify five phytoconstituents of pharmacological relevance, protocatechuic acid, syringic acid, kaempferol, apigenin and diosmetin[2,3]. These markers were specifically selected for their known antioxidant, anti-inflammatory and antimicrobial properties, all of which are essential for wound healing. Protocatechuic acid and syringic acid reduce oxidative stress by scavenging reactive oxygen species[1,4,5].

Kaempferol and apigenin downregulate pro-inflammatory cytokines such as TNF-α and IL-6, thereby preventing prolonged inflammation and diosmetin contributes both anti-inflammatory and antimicrobial effects[6]. Validation of these phytoconstituents by analytical methods ensures consistency in reproducibility, accuracy in dosage and reliability in therapeutic effects, which is important for the development of evidence-based herbal products[7,8]. In addition to chemical standardization, it is important to conduct *in vivo* efficacy testing. The Zebrafish (Danio rerio) model has recently been recognized as a robust preclinical model for studies on wound healing, owing to its genetic resemblance to humans, its optical transparency that facilitates real-time monitoring and its exceptional regenerative abilities[9,10].

In this study, we used two zebrafish injury models: a superficial scratch assay and tail fin transection, to thoroughly assess wound healing. The polyherbal gel was evaluated against 1% silver sulfadiazine, a clinically recognized topical standard[11]. The primary outcome measures included the rate of wound closure, the process of re-epithelialization, the inflammatory response and collagen deposition. Together, these factors provided substantial insights into the therapeutic efficacy[12].

Thus, this current study combines traditional pharmacological knowledge with contemporary analytical and biological validation. By merging phytochemical analysis, meticulous formulation and *in vivo* evaluation using zebrafish, it aims to create a reproducible and biocompatible polyherbal gel with significant wound-healing potential. In addition to demonstrating efficacy and safety, the results underscore the broader significance of polyherbal strategies in addressing the global demand for natural, sustainable and accessible therapies for wound care.

MATERIALS AND METHODS

Chemicals and Standards

All chemicals and reagents used were accurate in both formulation and analytical assessments. Certified reference standards for protocatechuic acid, syringic acid, kaempferol, apigenin and diosmetin (purity ≥98%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). These compounds served as external calibration standards for quantitative HPLC analysis. Chromatographic solvents of HPLC grade, including methanol, water and formic acid, were purchased from Merck (Darmstadt, Germany). Before use, solvents were passed through 0.22 µm nylon membranes and subjected to ultrasonication for degassing, aiming to reduce baseline variations, prevent bubble formation and safeguard the integrity of the column and detector system. The careful management of solvents and standards was executed to ensure analytical consistency and precision.

Plant Materials and Extract Preparation

Five medicinal plants traditionally used for wound management, *Trigonella foenum-graecum (fenugreek), Lawsonia inermis (henna), Basella alba (Malabar spinach), Peristrophe paniculata and Portulaca oleracea (purslane)*, were selected for this polyherbal formulation.

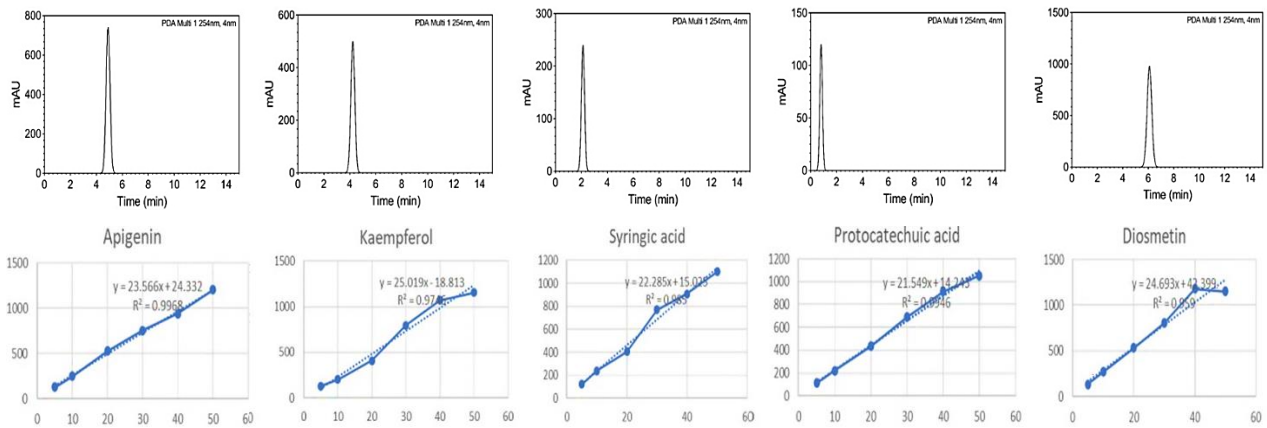

Figure 1: HPLC Chromatograms and Calibration curves of Standards

Table 1: Elution and Chromatographic Conditions of HPLC

Time (min)	% Solvent B	Notes
0–2	30%	Initial conditions, allowing for the separation of early-eluting compounds
2–10	Linear gradient from 30% to 90%	Increasing the organic phase to elute more retained compounds
10–12	90%	Holding a high organic concentration to ensure elution of all compounds
12–15	Re-equilibration at 30%	Returning to initial conditions to prepare the column for the next injection and ensure reproducibility

Table 2: Calibration values and statistical data of HPLC standards

Compound	Linear Range (µg/ml)	Slope	Intercept	R^2
Protocatechuic acid	5–50	23.01	1.82	0.9996
Syringic acid	5–50	24.02	2.45	0.9995
Kaempferol	5–50	25.09	2.33	0.9997
Apigenin	5–50	26.05	3.12	0.9996
Diosmetin	5–50	27.18	2.91	0.9996

Table 3: HPLC analysis of polyherbal gel

Compound	Retention Time (min)	Peak Area (mAU·min)	Estimated Concentration (µg/g)
Protocatechuic acid	0.83	118	4.8
Syringic acid	2.14	249	9.5
Kaempferol	4.25	500	19.2
Apigenin	4.92	745	28.6
Diosmetin	6.09	980	38.5

Plant materials were sourced from verified local suppliers and were verified by an experienced botanist. Voucher samples of each species were deposited in the institutional herbarium to ensure traceability and for future reference.

Upon collection, the raw materials were rinsed with distilled water to eliminate impurities, then shade-dried at 25 ± 2 °C for 7–10 days and were ground into a coarse powder using a mechanical grinder. Shade drying was chosen to maintain the integrity of heat-sensitive phytoconstituents. Each powdered sample underwent individual extraction by being macerated in a hydroalcoholic solvent blend (ethanol: water, 70:30 v/v) at ambient temperature for 72 hours, with intermittent stirring. The choice of a hydroalcoholic solvent was made because of its ability to extract a diverse array of phytochemicals with different polarities effectively. Following the maceration phase, the extracts were filtered and concentrated under reduced pressure utilizing a rotary evaporator. The resultant semi-solid residues were dried, stored in sealed containers at 4 °C and prepared for incorporation into the formulation. This procedure ensured uniformity in extract yield and preserved bioactivity prior to being incorporated into the gel.

Polyherbal Gel Formulation

In the formulation of the polyherbal gel, Carbopol 940 (1% w/w) was used as the gelling agent. Carbopol was dispersed in distilled water and allowed to hydrate overnight. Glycerin (2% w/w) was added as a humectant, with methylparaben (0.2%) and propylparaben (0.1%) included as preservatives. Triethanolamine was gradually added to neutralize the mixture and adjust the pH to approximately 6.5 ± 0.1, a level similar to the physiological pH of the skin, thereby reducing the risk of irritation. Each plant extract was included at a concentration of 5% w/w, resulting in a total herbal concentration of 25% in the gel. The extracts were thoroughly combined with the hydrated Carbopol base while stirring gently to avoid aeration. The formulated gel was packaged in sterile containers and kept at room temperature. Macroscopic characteristics (such as color, odor, consistency and homogeneity) were recorded before additional testing.

HPLC Analysis

Standard Solutions

Individual stock solutions were prepared at a concentration of 1 mg/mL in HPLC-grade methanol and subsequently serially diluted to create working concentrations ranging from 5 to 50 µg/mL. Calibration curves were established using triplicate injections at each concentration level. Regression analysis yielded calibration equations along with coefficients of determination (R^2).

Sample Preparation

To conduct the analysis, 1 g of the polyherbal gel was extracted using 10 mL of a methanol: water mixture (80:20, v/v) with the addition of 0.1% formic acid. The resulting mixture was vortexed for 10 minutes and then subjected to sonication for 30 minutes and then centrifuged at 5000 rpm for 15 minutes. The supernatant was filtered using 0.22 µm syringe filters before injection.

Chromatographic Conditions

The HPLC analysis was performed using a Shimadzu (Kyoto, Japan) system equipped with a photodiode array (PDA) detector. A C18 column (250 × 4.6 mm, 5 µm) was used for separation, which was maintained at a temperature of 30 °C. The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in methanol), delivered at a flow rate of 1.0 mL/min, following a gradient program. The Elution and Chromatographic conditions of HPLC are listed below in Table 1.

Detection was set at 254 nm. Peaks were identified by comparing retention times and UV spectra with authentic standards. Quantification of analytes in gel samples was performed by interpolating peak areas into the respective calibration equations. Regression analysis was applied to calculate the slope, intercept and coefficient of determination (R^2) for each calibration curve. The high R^2 values obtained (≥0.999) confirmed excellent linearity, supporting the accuracy of the quantification approach. Results were expressed as µg of compound per gram of gel.

Zebrafish Wound Healing Studies

Animal Maintenance and Ethics

Adult zebrafish (Danio rerio), 3–6 months old, were obtained from a certified breeding facility. Fish were

housed in 10 L aquaria at 28 ± 1 °C, with a pH range of 7.0–7.5, under a 14 h light/10 h dark cycle and maintained with continuous aeration. Animals were acclimatized for one week and fed commercial flakes and live Artemia. All procedures were approved by the Institutional Animal Ethics Committee and conformed to international welfare guidelines.

Experimental Groups

Fish (n = 10–15/group) were randomly assigned to:

Control group – uninjured, untreated.

Negative control – injured, untreated.

Standard group – injured, treated with 1% silver sulfadiazine.

Treatment group – injured, treated with polyherbal gel.

Topical applications (5–10 µL) were administered once daily for 7 days.

Wound Induction

Two reproducible injury models were employed:

• Superficial scratch: Linear epidermal wounds were made on the flank with sterile needles while the fish were under MS-222 anesthesia (150 mg/L).

• A portion of the caudal fin, roughly 30%, was excised using a sterile scalpel while observed under a stereomicroscope.

Following the procedures, fish were placed in recovery tanks and monitored until they completely recuperated.

Monitoring and Analysis

The healing process was documented by daily imaging using a stereomicroscope. The wound areas were measured with ImageJ software. Wound closure was calculated as follows:

$$Wound\ closure\ \% = (A_0 - A_t)/A_0 \times 100\%$$

(where A_0 represents the initial wound area (Day 0) and A_t signifies the wound area at time t)

In the case of tail transection, the growth of regenerative tissue was quantified. After Day 7, the fish were euthanized using an overdose of MS-222 and the caudal fins were collected for histological analysis.

Histopathology

Fin tissues were fixed in 10% neutral-buffered formalin, subsequently dehydrated with a series of ethanol solutions, cleared with xylene and embedded in paraffin. Sections with a thickness of 4–5 µm were cut and stained using hematoxylin and eosin (H&E). A microscopic evaluation was performed to analyze re-epithelialization, infiltration of inflammatory cells, collagen alignment and the overall organization of the tissue.

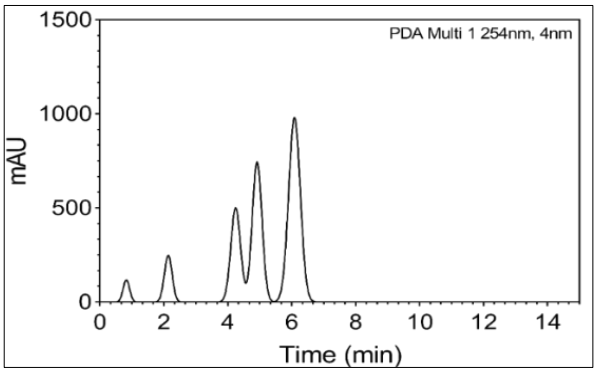

Figure 2: HPLC Chromatogram of polyherbal gel

Statistical Analysis

All the quantitative data were expressed as mean ± Standard Deviation. Differences among the groups were evaluated using one-way ANOVA followed by Tukey's post hoc test. A p-value of <0.005 was considered to be statistically significant. All the analyses were performed using SPSS software (IBM Corp., Armonk, NY, USA).

RESULTS

HPLC Method Development and Standard Calibration

A robust and optimized reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed for the first time to simultaneously identify and quantify five bioactive constituents in a polyherb gel formulation: protocatechuic acid, syringic acid, kaempferol, apigenin and diosmetin. Chromatographic separations were completed on a C18 column (250 mm × 4.6 mm, 5µm) at 30 °C and a photodiode array (PDA) detector was used. Excellent peak resolution and reproducibility were achieved across multiple runs. The mobile phase consisted of Solvent A (water with 0.1% formic acid) and Solvent B (methanol with 0.1% formic acid), which was perfected by a gradient elution technique. The program started with 30% B (0-2 min), which increased linearly to 90% B (2-10 min), maintained at 90% (10-12 min) and decreased to 30% B to recharge the column (12-15 min). This optimized gradient provided effective baseline separation of the analytes with insignificant peak tailing.

The protocatechuic acid (0.82 min), syringic acid (2.13 min), kaempferol (4.24 min), apigenin (4.91 min) and diosmetin (6.10 min) standards were well resolved with reproducible retention times (tR). Each compound displayed distinct UV absorbance maxima on the PDA detector, aligning with reported reference spectra, thus confirming their identities. Representative chromatograms showed sharp, symmetrical peaks with clear baseline separation, validating the method's suitability for complex phytochemical analysis. Quantitative validation was performed by constructing calibration curves for each analyte across six concentration levels (5–50 µg/mL). Calibration plots were generated by correlating peak area (mAU·min) with concentration (µg/mL). All analytes exhibited excellent linearity, with regression coefficients (R^2) consistently greater than 0.999, confirming the method's accuracy, precision and reliability for quantitative assessment.

Table 2 and Figure 1 present the calibration values and statistical data for the HPLC standards. These calibration profiles demonstrated strong analytical performance, using this HPLC method as a reliable tool for simultaneous quantification of multiple phytoconstituents in polyherbal formulations. These findings provide a validated foundation for subsequent formulation analysis and *in vivo* evaluation.

Quantitative Detection of Phytochemicals in Polyherbal Gel

The protocol was used for the analysis of extract samples from the polyherbal gel formulation after a successful validation using the HPLC method. The gel extract chromatogram obtained exhibited 5 major peaks, with retention times that matched those of the reference

standards, confirming the presence of the key phytoconstituents. In addition, peaks were corroborated through the co-injection of standards and analysis of the spectra by a PDA detector and co-injection confirmed the unambiguous allocation of the peaks through spectral overlay.

The regression equations from a set of calibration curves were used for quantitative analysis. The amounts of analyzed phytochemicals were calculated and are as follows protocatechuic acid (4.8 µg/g at tR 0.83 min); syringic acid (9.5 µg/g at tR 2.14 min); kaempferol (19.2 µg/g at tR 4.25 min); apigenin (28.6 µg/g at tR 4.92 min); and diosmetin (38.5 µg/g at tR 6.09 min). Of the five, the highest concentration was observed for diosmetin, whereas the lowest was for protocatechuic acid. These results of the HPLC analysis of polyherbal gel are outlined in Table 3 below.

Figure 2 above depicts the HPLC chromatogram of the polyherbal gel. The results ascertained that the phytochemicals were successfully incorporated into the gel formulation and remained chemically stable. This analytical validation provides an ideal quality control benchmark for the polyherbal gel, promoting its reproducibility as a standardized therapeutic product.

In-vivo Wound Healing – Mechanical Injury Model

The wound-healing activity of the polyherbal gel was initially demonstrated in zebrafish using the superficial mechanical scratch model. A linear injury model was established and wound healing was observed daily using stereomicroscopy. Further analysis was conducted using ImageJ software. The wound size at day 0 was considered 100% and healing rates were expressed as the percentage reduction.

The polyherbal gel-treated model demonstrated faster wound closure at all time points tested compared with the untreated control and the control treatment (1% silver sulfadiazine). On day 1, mean wound closure in the gel-treated fish had increased to 15.1 ± 2.4%, which was significantly higher than the negative control (5.2 ± 1.3) and the positive treated group (10.5 ± 2.2). This early advantage was sustained throughout the study. By Day 3, wound closure for the polyherbal gel therapy was 47.6% ± 3.5 in contrast to 35.3% ± 3.8 for the standard treatment and 12.7% ± 2.5 for the negative control. Closure at D5 was 70.3% ± 3.9 in the gel group, compared to 58.4% ± 4.6 and 20.4% ± 3.1 in the standard and control groups, respectively.

At the end of the study on Day 7, near-complete wound closure of 88.7% ± 2.7 was achieved by the polyherbal gel. By contrast, the control group closure was only 30.6% ± 4.2, whereas the normal treatment group closure was 75.9% ± 3.2. Furthermore, statistical analysis with one-way ANOVA followed by a Tukey's post hoc test proved that the differences in wound closure between the gel groups and the negative control were statistically significant ($p < 0.01$). All these values taken together reflect the fact that the polyherbal gel not only promotes faster rates of contraction but is more effective even compared to the standard silver sulfadiazine therapy. The results obtained from the Zebrafish wound healing analysis using the mechanical injury method are presented in Table 4 and Figure 3 below.

In-vivo Wound Healing – Tail Fin Transection Model

Additionally, the zebrafish under tail fin transection model has been utilized to assess the regenerative potential of the polyherbal gel. In contrast to surface injury systems, this assay directly interprets regenerative ability by tracking the regrowth of the amputated tissue. Partial incompletions of the caudal fin were generated and tissue regeneration was analyzed for about seven days. The 7-day consolidated results of the Zebra fish wound healing analysis in the Tail fin transection method are tabulated below in Table 5.

Figure 4 represents the Visual Morphology of Zebra Fish - Tail fin Transection method. Fin regrowth was highly improved in the polyherbal gel-treated group compared to the vehicle alone and the standard-treated group. From the 3rd day on, the reconstructed length of regenerated tissue was significantly higher in the gel group, suggesting an early pro-regenerative effect.

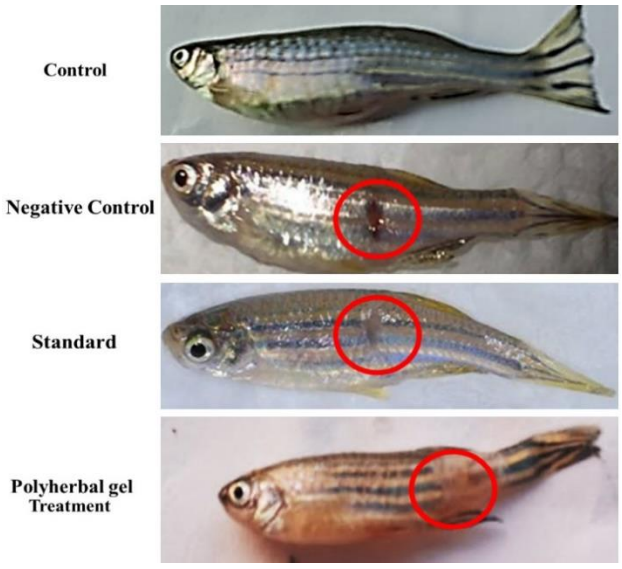

Figure 3: Visual Morphology of Zebra Fish -Mechanical injury method

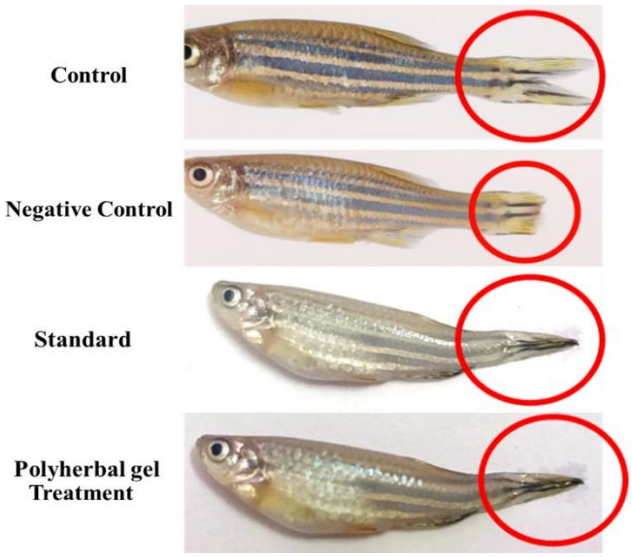

Figure 4: Visual Morphology of Zebra Fish -Tail fin Transection method

Table 4: Zebra fish wound healing analysis in the mechanical injury method

Group	Day 1 (%)	Day 3 (%)	Day 5 (%)	Day 7 (%)
Control	0	0	0	0
Negative Control	5.2 ± 1.3	12.7 ± 2.5	20.4 ± 3.1	30.6 ± 4.2
Standard	10.5 ± 2.2	35.3 ± 3.8	58.4 ± 4.6	75.9 ± 3.2
Polyherbal Gel	15.1 ± 2.4	47.6 ± 3.5	70.3 ± 3.9	88.7 ± 2.7

Table 5: Zebra fish wound healing analysis in the Tail fin transection method

Group	Day 1 (%)	Day 3 (%)	Day 5 (%)	Day 7 (%)
Control	0%	0%	0%	0%
Negative Control	10.5 ± 2.1%	26.2 ± 2.8%	41.8 ± 3.4%	55.4 ± 4.1%
Standard	18.7 ± 2.5%	52.3 ± 3.2%	72.6 ± 3.9%	90.2 ± 2.7%
Treatment	20.1 ± 2.7%	58.5 ± 3.4%	77.3 ± 3.2%	94.5 ± 2.1%

On Day 7, fish treated with the polyherbal gel displayed near-complete regeneration with a 94.5% ± 2.1 closure of the wound. In comparison, the standard treatment group (1% silver sulfadiazine) exhibited 90.2% ± 2.7 closure, while the negative control group (untreated fish) showed only 55.4% ± 4.1, reflecting limited natural recovery. As expected, the uninjured control group recorded 0.0% ± 0.0 closure, serving as the baseline reference. Statistical analysis confirmed that wound closure in the polyherbal gel group was significantly higher than both the standard and untreated controls ($p < 0.05$).

In contrast, the standard treatment group (1% silver sulfadiazine) showed 90.2% ± 2.7 closure, while the deprivation group (untreated fish) exhibited only 55.4% ± 4.1 closure, indicating a reduced ability for natural recovery. The uninjured control group had 0.0% ± 0.0 closure as would be expected (baseline reference).

Statistical analysis confirmed that wound closure in the polyherbal gel group was significantly higher than both the standard and untreated controls ($p < 0.05$). Collectively, these findings highlight the superior regenerative potential of the polyherbal gel. The incorporated phytochemicals not only accelerated wound contraction but also stimulated tissue remodeling and structural regrowth, enabling more efficient recovery than the conventional clinical standard. This enhanced regenerative response serves as a therapeutic strategy for wound repair and tissue regeneration.

Microscopic Regeneration Assessment

Microscopic evaluation provided compelling evidence of the regenerative efficacy of the polyherbal gel. Daily imaging revealed progressive and well-organized fin regrowth, with near-complete restoration of fin rays and pigmentation by Day 7. In contrast, untreated controls exhibited incomplete and irregular regeneration, accompanied by persistent inflammation and a disorganized tissue structure. Histopathological analysis further substantiated these findings, where uninjured controls displayed a normal fin architecture with intact epithelium, well-defined dermis and organized fin rays. Negative controls (injured, untreated) showed disrupted epidermis, heavy inflammatory infiltration, irregular or absent collagen deposition and truncated fin rays, reflecting poor healing. Standard treatment (1% silver sulfadiazine) resulted in partial epithelial reformation, moderate collagen alignment and reduced, yet persistent, inflammation, indicating incomplete tissue repair.

The polyherbal gel group exhibited the most extensive regeneration, with seamless re-epithelialization, compact and well-aligned collagen fibers and fully restored fin rays. Minimal inflammatory infiltration was observed,

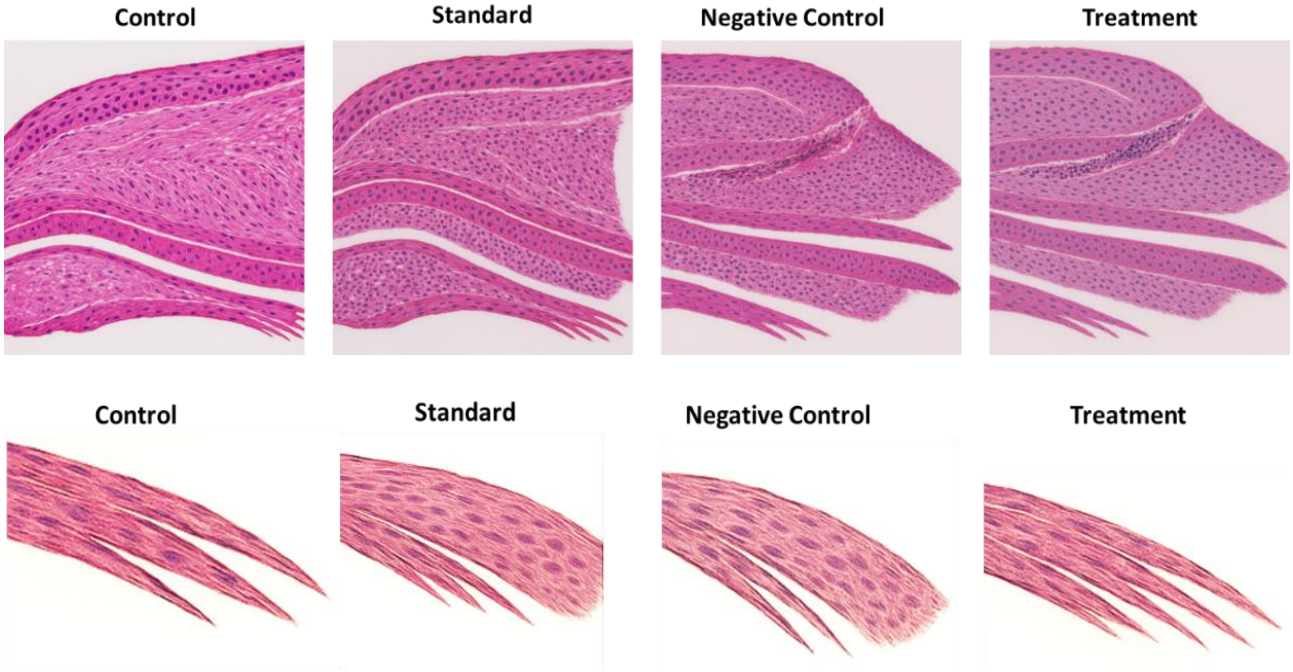

Figure 5: Histopathological evaluation of Zebra fish wound sites using H & E staining

highlighting both its regenerative and anti-inflammatory effects. Collectively, these microscopic findings, as demonstrated in Figure 5, confirm the gel's ability to promote high-quality structural and functional tissue restoration.

DISCUSSION

This current study developed and validated a polyherbal gel prepared from *Trigonella foenum-graecum*, *Lawsonia inermis*, *Basella alba*, *Peristrophe paniculata* and *Portulaca oleracea*, selected based on their ethnomedicinal relevance and reported pharmacological activities in wound healing. The formulation was rigorously evaluated through phytochemical standardization by RP-HPLC and *in vivo* validation in zebrafish wound models, providing both chemical and biological evidence of efficacy[13-16].

A persistent limitation in herbal medicine research is the lack of reproducible standardization, which impedes clinical acceptance. To address this, five marker phytoconstituents —protocatechuic acid, syringic acid, kaempferol, apigenin and diosmetin —were quantified with excellent precision and reproducibility ($R^2 > 0.999$). Chemical fingerprinting confirmed their stability and presence in the gel, ensuring quality control across batches[17-19]. Additionally, diosmetin, apigenin and kaempferol were present in higher concentrations, consistent with their known anti-inflammatory, antioxidant and antimicrobial properties, which are crucial for wound repair.

The zebrafish model was used as a translational platform due to its transparent integument, rapid regenerative potential and relevance to studying tissue repair dynamics[20-22]. In both mechanical injury and tail fin transection assays, the polyherbal gel markedly accelerated wound closure compared to negative controls and even surpassed the standard comparator, silver sulfadiazine. By Day 7, wound contraction reached 88.7% in the scratch model and 94.5% in the fin transection model, significantly higher than untreated controls and marginally superior to silver sulfadiazine ($p < 0.05$)[23].

Histological analysis provided structural confirmation of these macroscopic observations. Polyherbal gel-treated tissues demonstrated seamless re-epithelialization, organized collagen deposition, minimal inflammatory infiltration and complete restoration of the fin rays. In contrast, negative controls exhibited persistent inflammation and poorly aligned dermal structures, whereas silver sulfadiazine-treated samples showed moderate but incomplete tissue repair[24-29]. This highlights the gel's ability to not only enhance wound closure but also promote deeper structural regeneration.

The phytochemical composition supports the mechanistic plausibility of these effects. Protocatechuic and syringic acids act as potent antioxidants, mitigating reactive oxygen species that delay wound repair[29,30]. Apigenin and kaempferol are well-documented suppressors of pro-inflammatory cytokines, such as TNF-α and IL-6, while enhancing TGF-β signaling, which is crucial for fibroblast proliferation and extracellular matrix remodeling[31]. Diosmetin provides additional antimicrobial activity, potentially lowering infection risks while modulating inflammation[32]. Collectively, these compounds act in a synergistic, multi-targeted manner to regulate oxidative stress, inflammation, microbial colonization and collagen synthesis, which are key processes spanning all phases of wound healing.

Although silver sulfadiazine remains the clinical gold standard due to its antimicrobial efficacy, its drawbacks, including delayed epithelialization, cytotoxicity and the development of resistance, limit its prolonged use. In contrast, the polyherbal formulation exhibited a balanced healing profile without observable toxicity or irritation, making it a promising alternative for sustained or over-the-counter use. The polyherbal approach also carries broader therapeutic implications. By incorporating multiple herbs, the formulation covers diverse mechanistic pathways, thereby minimizing the limitations of single-agent therapy. Such synergistic interactions, combined with favourable biocompatibility, strengthen its translational potential as a safe, natural and accessible wound care product.

Moreover, several limitations must be noted. The relative contribution of each plant extract or individual compound was not dissected, leaving the precise molecular drivers of the effect unidentified. Future studies employing pathway-specific inhibitors or genetic models could elucidate these mechanisms[33-35]. While zebrafish provide valuable insights into regeneration, validation in mammalian models and eventual clinical trials are essential before therapeutic translation[36-38]. Stability studies were also beyond the scope of this work, yet they are critical to ensuring shelf life and potency over time. Optimization of concentration, dosing intervals and delivery systems could further refine its clinical utility.

CONCLUSION

This study demonstrates the successful development of a polyherbal gel containing *Trigonella foenum-graecum*, *Lawsonia inermis*, *Basella alba*, *Peristrophe paniculata* and *Portulaca oleracea*, with phytochemical validation confirming the stability of key bioactive compounds. *In vivo* evaluation in zebrafish models revealed significant wound closure, superior tissue regeneration and restored histological architecture, with outcomes comparable to or superior to those of silver sulfadiazine. The enhanced healing effects are attributed to the synergistic anti-inflammatory, antioxidant and tissue-remodeling actions of the phytoconstituents. Collectively, these findings establish the gel as a safe and effective herbal formulation for wound healing, warranting further validation in mammalian models and clinical settings.

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