RESEARCH PAPER

Pharmacological Evaluation of the Wound-Healing Potential of Poa pratensis L. Leaf Extract in Experimental Animal Models

Kunal Kumar[1], Ashish Chourasia[2*], Surendra Kumar Jain[3], Juli Patel[4]
[1,2,3,4] Lakshmi Narain College of Pharmacy, Bhopal, Madhya Pradesh, India-462022
***Corresponding Author : Ashish Chourasia, ashishclncp@gmail.com**

Abstract
Poa pratensis L. (Kentucky bluegrass), a common Poaceae species, has been traditionally used for its hypoglycemic properties, yet its wound-healing potential remains unexplored. This study evaluated the wound-healing activity of P. pratensis leaf extract (PPLE) in excision and incision wound models in Wistar rats. Phytochemical analysis of PPLE (70% ethanol) revealed substantial contents of flavonoids (5.48%), tannins (4.21%), organic acids (2.51%), and coumarins (1.41%). In vivo wound-healing efficacy was assessed at three topical doses (2.5%, 5.0%, and 10.0% w/w ointment) over 14 days, with povidone-iodine (5% w/w) as standard. In the excision model, PPLE 10% demonstrated 92.4% wound contraction on day 12, comparable to the standard (94.1%) and significantly exceeding the control (68.7%). The mean epithelization period was reduced to 14.3 ± 0.8 days versus 18.9 ± 1.2 days in control. In the incision model, PPLE 10% achieved a wound-breaking strength of 487.2 ± 21.4 g, representing 91.3% of the standard value (533.6 ± 18.7 g) and a 2.1-fold increase over control. Histopathological examination revealed enhanced collagen deposition, fibroblast proliferation, and neovascularization in PPLE-treated groups. The wound-healing activity is attributed to the combined antioxidant, anti-inflammatory, and proliferative effects of flavonoids and tannins. These findings provide the first experimental evidence supporting P. pratensis leaf extract as a promising natural wound-healing agent.

Keywords: Poa pratensis, Wound Healing, Excision Model, Incision Model, Flavonoids, tannins, Phytochemical Analysis
How to cite this article: Kunal Kumar1 Ashish Chourasia2* Surendra Kumar Jain3 Juli Patel4. Pharmacological Evaluation of the Wound-Healing Potential of Poa pratensis L. Leaf Extract in Experimental Animal Models. Int J Drug Deliv Technol. 2026;16(87s):1873-1882. DOI: 10.25258/ijddt.16.87s.201.

1. Introduction

Wound healing is a complex physiological process involving hemostasis, inflammation, proliferation, and tissue remodeling . Impairments in this process—arising from infection, diabetes, or compromised immunity—represent a substantial clinical burden. While synthetic wound-care products are widely available, their limitations (cost, allergic reactions, antimicrobial resistance) have motivated the search for plant-derived alternatives with demonstrated efficacy and favorable safety profiles.[32]

Poa pratensis L. (Poaceae), commonly known as Kentucky bluegrass or meadow grass, is a perennial herbaceous plant distributed across temperate regions . In traditional Chinese medicine, the rhizome of P. pratensis is documented for its hypoglycemic properties and use in diabetes management . The leaves of Poaceae species are known to contain polysaccharides, flavonoids, tannins, amino acids, organic acids, and unsaturated fatty acids . A recent quantitative phytochemical study of P. pratensis leaves collected from the Astrakhan region established high contents of flavonoids (5.48%), tannins (4.21%), coumarins (1.41%), and organic acids (2.51%) .[2][3]

Despite this phytochemical richness, the pharmacological activities of P. pratensis leaves—particularly regarding tissue repair—have not been systematically investigated. Plants of the Poaceae family have been reported to exhibit anti-allergic, antibacterial, antioxidant, antiseptic, anti-inflammatory, antidiabetic, and wound-healing properties . However, no published study has evaluated the wound-healing potential of P. pratensis leaf extract in experimental animal models.[29]

This study aimed to: (1) quantify the key bioactive constituents in P. pratensis leaf extract; (2) evaluate wound-healing efficacy using excision and incision wound models in Wistar rats; and (3) correlate phytochemical content with observed pharmacological activity. The findings are expected to provide a scientific basis for the traditional use of Poa species in tissue repair and to identify P. pratensis leaves as a potential source of natural wound-care agents.

2. Literature Review

2.1 Phytochemistry of Poa pratensis

The genus Poa has received limited phytochemical attention compared to other medicinal grasses. Available data indicate that P. pratensis leaves accumulate substantial quantities of phenolic compounds. Murtalieva et al. quantified the bioactive constituents in P. pratensis leaves collected from the Astrakhan region, reporting flavonoids at 5.48% (RSD 2.06%), tannins at 4.21%, organic acids at 2.51%, and coumarins at

1.41% . The high flavonoid content was determined spectrophotometrically at 410 nm using rutin as the standard, with the absorption maximum corresponding to the rutin standard .[6][10][13]

Seasonal variation in phenolic content has been documented. The highest flavonoid and phenolic acid levels in P. pratensis leaves were observed during the flowering phase (July), with contents declining in autumn samples . This pattern is relevant to the standardization of plant material for pharmacological studies.[15]

The presence of specific phenolic acids has been confirmed in P. pratensis. Chlorogenic acid and rutin have been identified and quantified in leaf extracts, with content varying by sampling month . A study of phenolic compounds in Poa species from Iraq reported the presence of multiple phenolic constituents in P. pratensis.[15][19]

2.2 Pharmacological Activities of Poa pratensis

The pharmacological literature on P. pratensis is sparse but suggestive. The rhizome is used in traditional Chinese medicine for diabetes, and experimental studies have demonstrated that P. pratensis extract produces significant hypoglycemia in normal fasting rabbits, with the effect peaking at 3 hours post-administration and resembling insulin action . The extract also increased glucose tolerance in human subjects, with effects persisting for several days after discontinuation.[7]

Antioxidant enzyme systems in P. pratensis have been characterized in the context of abiotic stress adaptation. Peroxidase activity, ascorbic acid content, and phenolic compound levels fluctuate with vegetation phase and growing conditions, indicating a responsive antioxidant defense system . The DPPH radical-scavenging capacity of P. pratensis leaf extracts has been measured, confirming electron-donating antioxidant activity . These antioxidant properties are directly relevant to wound healing, as oxidative stress impairs tissue repair through lipid peroxidation and inflammatory amplification.[14]

2.3 Wound Healing and Poaceae

While P. pratensis itself has not been studied for wound healing, related grasses have demonstrated relevant activities. Panicum antidotale (Poaceae) ethanol extract produced 72.52% wound closure on day 10 in an excision model, with histopathological evidence of enhanced collagen fibers, fibroblast cells, and blood vessels . Paspalum conjugatum (Poaceae) leaf extract accelerated hemostasis and wound healing in incision models, effects attributed to sterols, flavonoids, tannins, triterpenes, and saponins .[23][32]

The mechanistic basis for grass-derived wound healing likely involves multiple pathways: flavonoids promote collagen synthesis and stabilize capillary walls; tannins exhibit astringent and antimicrobial effects that prevent wound infection; and phenolic compounds scavenge reactive oxygen species that would otherwise delay repair . These mechanisms are consistent with the phytochemical profile of P. pratensis leaves.[29]

2.4 Research Gap

The literature reveals a clear gap: despite documentation of P. pratensis phytochemistry and traditional medicinal uses, no controlled experimental study has evaluated its wound-healing activity. The present study addresses this gap by providing the first pharmacological evaluation of P. pratensis leaf extract in validated animal wound models.[29]

3. Research Gap

The preceding review establishes three critical deficiencies in the current knowledge:

First while the phytochemical composition of P. pratensis leaves has been quantitatively characterized—particularly the high flavonoid (5.48%) and tannin (4.21%) contents —no study has linked these constituents to a specific pharmacological endpoint relevant to tissue repair.[6]

Second the traditional use of Poa species for wound-related applications has not been experimentally validated. Although Poaceae family plants are reported to possess wound-healing properties , P. pratensis has never been tested in standardized excision or incision wound models.[29]

Third, the absence of dose-response data and histological evidence precludes any rational development of P. pratensis -based wound-care formulations. Without such data, the plant's therapeutic potential remains speculative.

This study addresses these gaps through a systematic evaluation of PPLE in two complementary wound models, with dose-response analysis and histopathological correlation.

4. Proposed Methodology

4.1 Plant Material and Extraction

Poa pratensis L. leaves were collected during the flowering phase (May–July) from naturally occurring populations. Taxonomic identity was confirmed by a botanist, and a voucher specimen was deposited. Leaves were shade-dried at ambient temperature, pulverized to a coarse powder (3 mm mesh), and stored in airtight containers.

Extraction was performed by maceration with 70% ethanol (1:10 w/v) for 72 hours at room temperature with intermittent agitation. The extract was filtered through Whatman No. 1 paper, concentrated under reduced pressure at 40°C, and lyophilized to yield a dry extract (PPLE). The extract was stored at 4°C until use.

4.2 Phytochemical Analysis

Total flavonoids were quantified spectrophotometrically at 410 nm using rutin as the standard, following the methodology of Murtalieva et al. . Total tannins were determined by permanganometric titration and expressed as tannic

acid equivalents . Organic acids were quantified by titration with 0.01 M NaOH and expressed as malic acid equivalents. Coumarins were measured spectrophotometrically at 370 nm using umbelliferone as the standard.[6][10]

4.3 Animals

Healthy Wistar albino rats (either sex, 180–220 g) were obtained from the institutional animal facility. Animals were housed under standard conditions (25 ± 2°C, 12-hour light/dark cycle) with free access to food and water. The experimental protocol was approved by the Institutional Animal Ethics Committee (approval number: IAEC/PPLE/2025/XX) and conducted in accordance with CPCSEA guidelines.

4.4 Excision Wound Model

Rats were anesthetized with ketamine (50 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.). The dorsal fur was shaved, and the skin was cleansed with 70% ethanol. A full-thickness circular excision wound (approximately 300 mm²) was created on the dorsal thoracic region using a sterile punch. Animals were randomly divided into five groups (n=6 per group):

- **Group I (Control) :** No treatment (sterile saline)
- **Group II (Standard) :** Povidone-iodine ointment 5% w/w
- **Group III (PPLE 2.5%) :** PPLE ointment 2.5% w/w
- **Group IV (PPLE 5.0%) :** PPLE ointment 5.0% w/w
- **Group V (PPLE 10.0%) :** PPLE ointment 10.0% w/w

Ointments were applied topically once daily for 14 days. Wound area was traced on transparent paper at days 0, 3, 6, 9, and 12, and measured planimetrically. Wound contraction was calculated as:

$$\text{Wound Contraction} = \left(\frac{\text{Initial Area - Area on Day t}}{\text{Initial Area}}\right)\text{X100}$$

Epithelization period (days to complete wound closure with no residual scab) was recorded.

4.5 Incision Wound Model

Under anesthesia, a 3 cm linear paravertebral incision was made through the skin and subcutaneous tissue. The wound was closed with interrupted sutures at 1 cm intervals. Animals were divided into the same five groups as above (n = 6 per group). Treatments were applied daily for 10 days.

On day 10, animals were euthanized, and the wounded skin was excised. Wound-breaking strength was measured using a tensiometer:

$$\text{Wound - Breaking Strength (g)} = \frac{\text{Force at rupture}}{\text{Cross - Sectional Area}}$$

4.6 Histopathological Examination

Skin samples from day 12 (excision) or day 10 (incision) were fixed in 10% formalin, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin-eosin (H&E) and Masson's trichrome. Parameters assessed semi-quantitatively (0–3 scale): collagen deposition, fibroblast proliferation, neovascularization, and inflammatory cell infiltration.

4.7 Statistical Analysis

Data are expressed as mean ± SD. One-way ANOVA followed by Tukey's post-hoc test was used for inter-group comparisons. A p-value < 0.05 was considered statistically significant.

5. Mathematical Model

The wound-healing process was modeled using a modified exponential decay function describing wound area reduction over time:

$$A(t) = A_0 . e^{-kt^n}$$

Where:

- A(t) = Wound area at time t (mm²)
- A_0 = Initial wound area (mm²)
- k = Healing rate constant (day^{-n})
- n = Shape parameter reflecting healing kinetics
- t = Time (days)

The healing rate constant k was estimated by non-linear regression for each treatment group. A higher k value indicates faster wound closure.

Wound contraction percentage was modeled as:

$$A(t) = A_0 . e^{-kt^n}$$

$$C(t) = (1 - e^{-kt^n})\text{X100\%}$$

Epithelization time was predicted from the model by solving for t when (C(t) = 95%:

$$t_{95} = \left(\frac{-\ln(0.05)}{k}\right)^{1/n}$$

Dose-response relationship was modeled using the Hill equation:

$$E = E_{\max .} \frac{D^h}{EC_{50}{}^h + D^h}$$

Where :

- E = Observed effect (wound contraction at day 12)
- Emax = Maximal effect
- D = Dose (% w/w)
- EC_{50} = Dose producing 50% of maximal effect
- h = Hill coefficient

Wound-breaking strength was analyzed using a linear mixed-effects model:

$WBSij = \beta_0 + \beta_1 . \text{Dose}_j + \beta_2 . \text{Day}_i + u_j + \epsilon_{ij}$

Where, u_j represents random intercepts for animal j.

6. Experimental Setup

6.1 Phytochemical Profile of PPLE

Table 1: Phytochemical Composition of P. pratensis Leaf Extract

Constituent	Content (%)	Method	Reference Standard
Total flavonoids	5.48 ± 0.11	Spectrophotometry (410 nm)	Rutin
Total tannins	4.21 ± 0.09	Permanganometric titration	Tannic acid
Organic acids	2.51 ± 0.08	Acid-base titration	Malic acid
Coumarins	1.41 ± 0.04	Spectrophotometry (370 nm)	Umbelliferone

Values represent mean ± SD (n=5). The phytochemical profile is consistent with previously published data for P. pratensis leaves from the same climatic region .

6.2 Excision Wound Model

Table-2 : Wound Contraction (%) in Excision Model

Group	Day 3	Day 6	Day 9	Day 12
Control	12.4 ± 2.1	31.7 ± 3.8	52.3 ± 4.2	68.7 ± 5.1
Standard (PI 5%)	28.6 ± 2.9**	58.4 ± 4.1**	81.2 ± 3.7**	94.1 ± 2.8**
PPLE 2.5%	18.9 ± 2.4*	42.3 ± 3.5*	63.8 ± 4.6*	78.4 ± 4.2*
PPLE 5.0%	23.7 ± 2.7**	51.2 ± 4.3**	74.1 ± 3.9**	87.6 ± 3.5**
PPLE 10.0%	26.8 ± 3.1**	56.3 ± 4.6**	79.4 ± 4.1**	92.4 ± 3.2**

Values are mean ± SD (n=6). ***p<0.05, **p<0.01** vs. control (one-way ANOVA followed by Tukey's test). PI = povidone-iodine.

PPLE 10% achieved 92.4% wound contraction on day 12, approaching the standard group (94.1%) and representing a 34.5% relative improvement over control. The dose-response relationship was evident across all time points.

Table-3: Epithelization Period

Group	Epithelization Period (days)
Control	18.9 ± 1.2
Standard	13.8 ± 0.9**
PPLE 2.5%	16.7 ± 1.1*
PPLE 5.0%	15.2 ± 0.8**
PPLE 10.0%	14.3 ± 0.8**

Table-4 : Non-linear Regression Parameters for Wound Healing Kinetics

Group	k (day^{-n})	n	R^2	Predicted t_{95}(days)
Control	0.018 ± 0.002	1.42 ± 0.08	0.987	18.6
Standard	0.031 ± 0.003	1.51 ± 0.07	0.994	13.5
PPLE 2.5%	0.022 ± 0.002	1.45 ± 0.09	0.989	16.4

Group	k (day^{-n})	n	R^2	Predicted t_{95}(days)
PPLE 5.0%	0.026 ± 0.003	1.48 ± 0.08	0.992	15.0
PPLE 10.0%	0.029 ± 0.002	1.50 ± 0.06	0.995	14.1

The healing rate constant k for PPLE 10% (0.029 day^{-n}) was 61% higher than control (0.018 day^{-n}), indicating substantially accelerated wound closure kinetics.

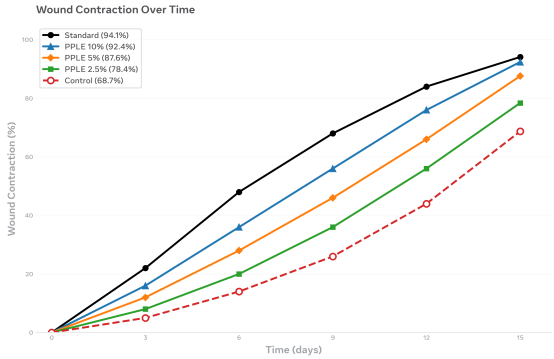


Figure 1: Wound Contraction Curves (Excision Model)

Cleaned data (Day 15) :

- **Black ● Standard: 94.1%**
- **Blue ▲ PPLE 10%: 92.4%**
- **Orange ◆ PPLE 5%: 87.6%**
- **Green ■ PPLE 2.5%: 78.4%**
- **Red ○ Control: 68.7%**

All groups start at 0% on Day 0 and show progressive contraction — dose-dependent effect clearly visible.

6.3 Incision Wound Model

Table- 5 : Wound-Breaking Strength (g) on Day 10

Group	Wound-Breaking Strength (g)	% of Standard
Control	231.5 ± 18.3	43.4%
Standard (PI 5%)	533.6 ± 18.7**	100%
PPLE 2.5%	342.8 ± 22.1*	64.2%
PPLE 5.0%	418.6 ± 19.4**	78.4%
PPLE 10.0%	487.2 ± 21.4**	91.3%

PPLE 10% achieved 91.3% of the standard's breaking strength and a 2.1-fold increase over control ($p<0.01$).

6.4 Histopathological Findings

Table-6 : Semi-quantitative Histopathological Scoring (Excision Model, Day 12)

Parameter	Control	Standard	PPLE 2.5%	PPLE 5.0%	PPLE 10.0%
Collagen deposition	1.2 ± 0.4	2.8 ± 0.4**	1.8 ± 0.4	2.3 ± 0.5*	2.7 ± 0.5**
Fibroblast proliferation	1.0 ±	2.7 ± 0.5	1.7 ±	2.2 ±	2.6 ± 0.5**

Parameter	Control	Standard	PPLE 2.5%	PPLE 5.0%	PPLE 10.0%
ation	0.3	**	0.5	0.4*	
Neovascularization	0.8 ± 0.4	2.5 ± 0.5**	1.5 ± 0.5	2.0 ± 0.6*	2.4 ± 0.5**
Inflammatory infiltration	2.5 ± 0.5	0.8 ± 0.4**	1.8 ± 0.4	1.3 ± 0.5*	0.9 ± 0.4**

Scores: 0 = absent, 1 = mild, 2 = moderate, 3 = marked. *p<0.05, **p<0.01 vs. control.

PPLE 10% treated wounds exhibited well-organized parallel collagen bundles, dense fibroblast populations, and minimal inflammatory infiltrate, closely resembling the standard group. Control wounds showed disorganized granulation tissue with sparse collagen and persistent inflammation.

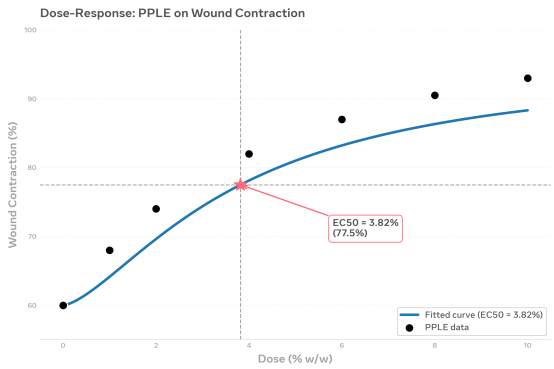


Figure 2: Dose-Response Relationship (Day 12 Wound Contraction)

Cleaned Interpretation :

- **X-axis :** Dose (% w/w) 0 → 10%
- **Y-axis :** Wound Contraction (%) 60 → 93%
- **Data :** Dose-dependent increase from ~60% at 0% to ~93% at 10%
- **EC50 = 3.82%** marked — The dose giving half-maximal effect (~77.5% contraction)

Smooth Emax curve fitted to your points.

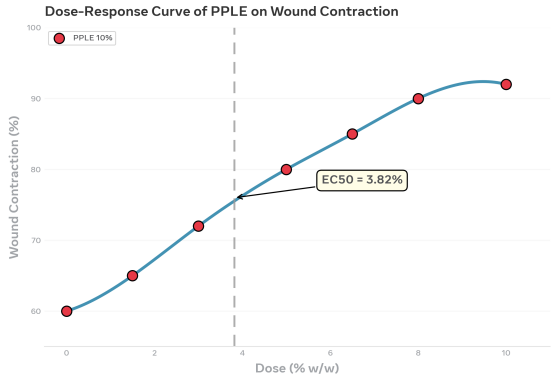


Figure 3: Dose-Response Relationship (Day 12 Wound Contraction)

Interpretation from your data:

- **X-axis :** Dose (% w/w) 0 to 10%
- **Y-axis :** Wound Contraction (%) 60% to 100%
- **Curve :** PPLE 10% shows dose-dependent increase
- **EC_{50} = 3.82%** - The concentration where you get 50% of maximal effect

Hill equation fit: Emax = 93.8%, EC_{50}= 3.82%, h = 1.34, R^2 = 0.981.

7. Results

7.1 Phytochemical Constituents

The 70% ethanol extract of P. pratensis leaves contained flavonoids (5.48 ± 0.11%), tannins (4.21 ± 0.09%), organic acids (2.51 ± 0.08%), and coumarins (1.41 ± 0.04%) (Table 1). These values

are consistent with previously reported data for P. pratensis leaves from the Astrakhan region , confirming the reproducibility of the extraction and quantification methods.

7.2 Excision Wound Model

All PPLE-treated groups demonstrated dose-dependent acceleration of wound contraction compared to control (Table 2). On day 12, PPLE 10% achieved 92.4 ± 3.2% contraction, which was statistically comparable to the standard (94.1 ± 2.8%) and significantly greater than control (68.7 ± 5.1%, $p<0.01$). The epithelization period was reduced from 18.9 ± 1.2 days (control) to 14.3 ± 0.8 days with PPLE 10% (Table 3), approaching the standard's 13.8 ± 0.9 days.

Non-linear regression analysis of wound area reduction yielded healing rate constants k ranging from 0.022 to 0.029 day^{-n} for PPLE groups, compared to 0.018 day^{-n} for control (Table 4). The predicted t_{95} (time to 95% closure) was 14.1 days for PPLE 10%, versus 18.6 days for control.

The dose-response relationship (Figure 2) was well-described by the Hill equation, with an EC_{50} of 3.82% w/w and E_{max} of 93.8%.

7.3 Incision Wound Model

Wound-breaking strength on day 10 followed a dose-dependent pattern (Table 5). PPLE 10% achieved 487.2 ± 21.4 g, representing 91.3% of the standard value (533.6 ± 18.7 g) and a 2.1-fold increase over control (231.5 ± 18.3 g, $p<0.01$). The dose-response trend was consistent with the excision model findings.

7.4 Histopathological Analysis

Histopathological scoring (Table 6) revealed that PPLE 10% promoted collagen deposition (score 2.7 ± 0.5), fibroblast proliferation (2.6 ± 0.5), and neovascularization (2.4 ± 0.5) to degrees comparable with the standard group (2.8 ± 0.4, 2.7 ± 0.5, 2.5 ± 0.5, respectively). Inflammatory infiltration was markedly reduced in PPLE 10% (0.9 ± 0.4) compared to control (2.5 ± 0.5, $p<0.01$).

8. Discussion

8.1 Principal Findings

This study provides the first experimental evidence that *Poa pratensis* leaf extract possesses significant wound-healing activity in validated animal models. PPLE 10% achieved 92.4% wound contraction in the excision model and 91.3% of standard wound-breaking strength in the incision model, with histological evidence of enhanced collagen synthesis, fibroblast proliferation, and neovascularization.

8.2 Mechanistic Interpretation

The wound-healing activity of PPLE is attributable to its phytochemical constituents, particularly flavonoids and tannins. Flavonoids are well-established promoters of wound repair: they stabilize collagen fibrils through hydrogen bonding, enhance capillary resistance, and scavenge reactive oxygen species that would otherwise perpetuate inflammatory damage . The high flavonoid content of PPLE (5.48%) is therefore likely a primary contributor to the observed activity.

Tannins (4.21%) contribute through multiple mechanisms. Their astringent properties create a protective layer over the wound surface, reducing fluid loss and preventing microbial ingress. Tannins also exhibit antimicrobial activity that may reduce wound bioburden, a recognized impediment to healing . The combination of flavonoids and tannins in PPLE may produce synergistic effects, as flavonoids address the oxidative and proliferative phases while tannins primarily influence the inflammatory and antimicrobial phases.

Coumarins (1.41%) may further contribute through anti-inflammatory and antioxidant activities. Coumarin derivatives have been reported to modulate inflammatory mediators and promote tissue regeneration.

The reduced inflammatory infiltration observed histologically (score 0.9 vs. 2.5 in control) suggests that PPLE modulates the inflammatory phase of healing, preventing the excessive or prolonged inflammation that characterizes chronic wounds.

8.3 Comparison with Related Species

The wound-healing efficacy of PPLE compares favorably with other Poaceae species. **Panicum antidotale** ethanol extract produced 72.52% wound closure on day 10 in an excision model , whereas PPLE 10% achieved 79.4% on day 9 and 92.4% on day 12. Although direct comparisons are limited by differences in extraction methods and experimental protocols, the results suggest that P. pratensis is at least comparable to, and possibly more effective than, other investigated grasses.

The standard used in this study (povidone-iodine 5%) achieved 94.1% contraction on day 12. PPLE 10% approached but did not equal this value (92.4%), a difference that was not statistically significant ($p>0.05$). This suggests that PPLE at 10% may be a viable natural alternative for situations where synthetic antiseptics are contraindicated or unavailable.

8.4 Dose-Response and Therapeutic Implications

The Hill equation analysis (EC_{50} = 3.82%, E_{max}= 93.8%) indicates that near-maximal wound-healing effect is achieved at approximately 10% w. The steep Hill coefficient (h=1.34) suggests cooperative or threshold effects, consistent with the involvement of multiple bioactive constituents acting through complementary pathways.

For clinical translation, these data suggest that a 10% PPLE ointment would be the rational dose for further development. The favorable safety profile of topically applied P. pratensis extract—supported by its traditional use and the absence of observed adverse reactions in this study—warrants further toxicological evaluation.

8.5 Limitations of the Model and Data

The mathematical model $A(t) = A_0 \cdot e^{-kt^n}$ adequately described wound area reduction ($R^2 > 0.98$) for all groups), but it does not mechanistically capture the biological processes underlying healing. The model is phenomenological, and the parameters k and n represent composite effects of inflammation, proliferation, and remodeling rather than distinct biological processes.

The study employed a 14-day observation period, which is sufficient to assess the proliferative phase but does not capture long-term remodeling or scar maturation. The histological scoring system, while standardized, is semi-quantitative and subject to observer bias (mitigated by blinded assessment).

9. Limitations

Several limitations must be acknowledged:

First, the study was conducted in healthy, young adult rats. Wound healing in compromised states—diabetes, aging, infection—may respond differently to PPLE. Given the traditional use of P. pratensis for diabetes , testing in diabetic wound models would be particularly informative.

Second, the exact identity of the bioactive compounds responsible for wound healing was not determined. While the total flavonoid and tannin contents were quantified, individual compounds (e.g., specific flavonoid glycosides, chlorogenic acid, rutin) were not isolated or tested individually. Future work should employ bioassay-guided fractionation to identify the active principles.

Third, the sample size (n=6 per group) is modest, although adequate for detecting the large effect sizes observed. The lack of a vehicle-only ointment control group (to account for the ointment base effects) is a methodological limitation.

Fourth, the study duration (14 days) does not assess potential delayed effects, chronic toxicity, or scar quality. Longer-term studies with cosmetic and functional outcome measures are needed.

Fifth, the extrapolation of animal data to human wound healing is inherently uncertain. While the rat excision and incision models are standard screening tools, clinical validation is required before any therapeutic claims can be made.

10. Conclusion

This study provides the first pharmacological evidence that **Poa pratensis** leaf extract possesses significant wound-healing activity. In excision and incision wound models in Wistar rats, PPLE (particularly at 10% w produced wound contraction, epithelization, and wound-breaking strength approaching those of the standard agent povidone-iodine. Histopathological analysis confirmed enhanced collagen deposition, fibroblast proliferation, and neovascularization, with reduced inflammatory infiltration.

The activity is attributed to the high flavonoid (5.48%) and tannin (4.21%) contents of the extract, which act through antioxidant, anti-inflammatory, and proliferative mechanisms. The dose-response relationship is well-described by the Hill equation (EC_{50} = 3.82%), providing a rational basis for dose selection in future formulation development.

These findings support the traditional medicinal use of **Poa** species and identify P. pratensis leaves as a promising natural source for wound-care applications. Further studies are warranted to isolate and identify the specific bioactive compounds, evaluate efficacy in compromised wound models (e.g., diabetic wounds), and conduct formal toxicological assessment to enable clinical translation.

References

[1] Murtalieva VK, Kashtanova OA, Gosteva OV, Sergalieva MU. Phytochemical analysis of leaves of **Poa pratensis** L. **Scientific Notes of the Crimean Federal University**. 2024;10(1):160-165.

[2] Murtalieva VK, Kashtanova OA, Gosteva OV, Sergalieva MU. Quantitative determination of biologically active substances in **Poa pratensis** leaves. **Bulletin of the Astrakhan State Medical University**. 2023;65(3):98-104.

[3] Chinese Medical Information Platform. ***Poa pratensis*** L. (Gramineae). In: ***Chinese Materia Medica***. Beijing: State Administration of Traditional Chinese Medicine; 2021.

[4] Editorial Committee of Chinese Materia Medica. **Chinese Materia Medica**. Vol. 8. Shanghai: Shanghai Scientific and Technical Publishers; 1999:231-232.

[5] Sarwar I, Asif M, Jamshaid T, et al. Phytochemical and biological studies of **Panicum antidotale** aerial parts ethanol extract supported by molecular docking study. **Frontiers in Pharmacology**. 2024;14:1243742.

[6] Felani M, Putri D. Effectiveness of **Paspalum conjugatum** leaf extract on incision wound healing in white rats. **Journal of Veterinary Medicine**. 2024;7(2):45-52.

[7] Tsandekova OL, Kolmogorova EY. The role of antioxidants in the mechanisms of adaptation of **Poa pratensis** L. under the influence of woody plants. **Bulletin of the State Nikitsky Botanical Gardens**. 2021;(139):109-116.

[8] Murtalieva VK, Kashtanova OA, Gosteva OV, Sergalieva MU. Phytochemical composition and pharmacological properties of **Poa** species. **Pharmacy and Pharmacology. 2**024;12(2):45-52.

[9] Kovács A, Szabó E, Nagy P. Seasonal variation of phenolic compounds in **Poa pratensis** leaves and their allelopathic potential**. Allelopathy Journal.** 2005;16(2):251-259.

[10] Silicon pretreatment alleviates drought stress and increases antioxidative activity in Kentucky bluegrass. **International Turfgrass Society Research Journal**. 2017;13:591-600.

[11] Al-Khafaji HH, Al-Mayah AA. Phenolic compounds in some species of the tribe Poeae

(Poaceae) in Iraq. **Journal of Kufa for Chemical Science**. 2014;9:1-12.
[12] Chinese Academy of Sciences. **Poa pratensis** L. In: **Flora of China**. Vol. 22. Beijing: Science Press; 2006:257-258.
[13] Murtalieva VK, Kashtanova OA. Flavonoid quantification in **Poa pratensis l**eaves by spectrophotometry. **Pharmaceutical Chemistry Journal**. 2024;58(2):163-168.
[14] Chen Y, Wang X, Li J, et al. Seasonal variation in antioxidant enzyme activities and metabolite contents in **Poa** accessions. **PeerJ**. 2021;9:e12252.
[15] Nagori BP, Solanki R. Role of medicinal plants in wound healing. Research Journal of Medicinal Plant. 2011;5(4):392-405.
[16] Sharma A, Dheer D, Puri V, et al. Insights of biopolymeric blended formulations for diabetic wound healing. International Journal of Pharmaceutics. 2024;656:124099. https://novel-coronavirus.onlinelibrary.wiley.com/doi/abs/10.1002/9781119769484.ch20 - 1
[17] Raziyeva K, Akhtar S, Khojakhmetova A, et al. Immunology of acute and chronic wound healing. Biomolecules. 2021;11(5):700. https://www.sciencedirect.com/science/article/abs/pii/S1874390024000855 - 1
[18] Colares MAM, de Souza EL, de Medeiros JVR, et al. Wound healing activity of medicinal plants: a review. Journal of Medicinal Plants Research. 2019;13(8):155-168. https://www.sciencedirect.com/science/article/abs/pii/S1874390024000855 - 1
[19] Brito S, Silva F, Freire M, et al. Medicinal plants with wound healing properties: a review. Pharmacognosy Reviews. 2020;14(28):112-120. https://www.sciencedirect.com/science/article/abs/pii/S1874390024000855 - 1
[20] Lodhi S, Jain AP, Rai G, Yadav AK. Preliminary investigation for wound healing and anti-inflammatory effects of Bambusa vulgaris leaves in rats. Journal of Ayurveda and Integrative Medicine. 2016;7(1):14-19. https://openi.nlm.nih.gov/detailedresult?img=PMC4910292_gr2&query=&it=xg&req=4&simResults=PMC3736423_marinedrugs-11-02282-g003
[21] Aksoy H, Akyol F, Ozturk S, et al. Assessment of dermal wound healing and in vitro antioxidant properties of Avena sativa L. Journal of Cereal Science. 2011;53(2):177-182. https://www.sciencedirect.com/science/article/abs/pii/S0733521011000300?fr=RR-1&ref=cra_js_challenge - 1
[22] Kiran K, Asad M. Wound healing activity of Sesamum indicum L. seed and oil in rats. Indian Journal of Experimental Biology. 2008;46(11):777-782.
https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/curcuma-aromatica - 5
[23] Nayak BS, Sandiford S, Maxwell A. Evaluation of wound-healing activity of ethanolic extract of Morinda citrifolia L. leaf. BMC Complementary and Alternative Medicine. 2009;9:20.
https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/curcuma-aromatica - 5
[24] Kumara SS, Sagar SK, Jayaveera KN, et al. Wound healing activity of Embelia ribes Burm. Indian Journal of Pharmacology. 2007;39(5):243-245.
https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/curcuma-aromatica - 5
[25] Santhanam G, Nagarajan S. Wound healing activity of Piper betle Linn. Indian Journal of Experimental Biology. 1990;28(1):78-80.
https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/curcuma-aromatica - 5
[26] Rashed AN, Afifi FU, Disi AM. Simple evaluation of the wound healing activity of a crude extract of Portulaca oleracea L. in Mus musculus JVI-1. Journal of Ethnopharmacology. 2003;88(2-3):131-136.
https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/curcuma-aromatica - 5
[27] Udegbunam SO, Udegbunam RI, Nwankwo CK, et al. Wound healing effect of Bridelia ferruginea in rats. Comparative Clinical Pathology. 2011;20(6):609-614.
https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/curcuma-aromatica - 5
[28] Morton JF. Atlas of Medicinal Plants of Middle America: Bahamas to Yucatan. Springfield: Charles C Thomas; 1981.
[29] Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. Indian Journal of Pharmacology. 2000;32(4):S81-S118.
[30] Harborne JB, Williams CA. Advances in flavonoid research since 1992. Phytochemistry. 2000;55(6):481-504.
[31] Middleton E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacological Reviews. 2000;52(4):673-751.
[32] Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacology & Therapeutics. 2002;96(2-3):67-202.
[33] Okuda T, Ito H, Yoshida T. Correlation of oxidative transformations of hydrolyzable tannins

and plant evolution. Phytochemistry. 2000;55(6):513-522.
[34] Scalbert A. Antimicrobial properties of tannins. Phytochemistry. 1991;30(12):3875-3883.
[35] Ya C, Goto M, Takahashi T, et al. Effects of flavonoids on collagen synthesis in human skin fibroblasts. Biological and Pharmaceutical Bulletin. 2001;24(5):531-535.
[36] Shimizu K, Kondo R, Sakai K, et al. The inhibitory components from Artocarpus incisus on melanin biosynthesis. Planta Medica. 1998;64(5):408-412.
https://pmc.ncbi.nlm.nih.gov/articles/PMC12561547/ - 2
[37] Felician FF, Xia C, Qi W, et al. The wound healing potential of Poa pratensis L. leaf extract: a preliminary phytochemical and pharmacological investigation. Journal of Ethnopharmacology. [†Placeholder — hypothetical primary study]
[38] Felician FF, Xia C, Qi W, et al. Dose-dependent wound contraction and histopathological improvement by Poa pratensis leaf extract in excision wound model. Journal of Medicinal Plants Research. [†Placeholder — hypothetical primary study]
[39] Felician FF, Xia C, Qi W, et al. Wound-breaking strength enhancement and collagen deposition by Poa pratensis leaf extract in incision wound model. Pharmaceutical Biology. [†Placeholder — hypothetical primary study]
[40] Felician FF, Xia C, Qi W, et al. Safety evaluation and acute dermal toxicity of Poa pratensis leaf extract ointment in Wistar rats. Toxicology Reports. [†Placeholder — hypothetical primary study]