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Phytochemical Screening And Pharmacological Evaluation Of Pergularia Daemia

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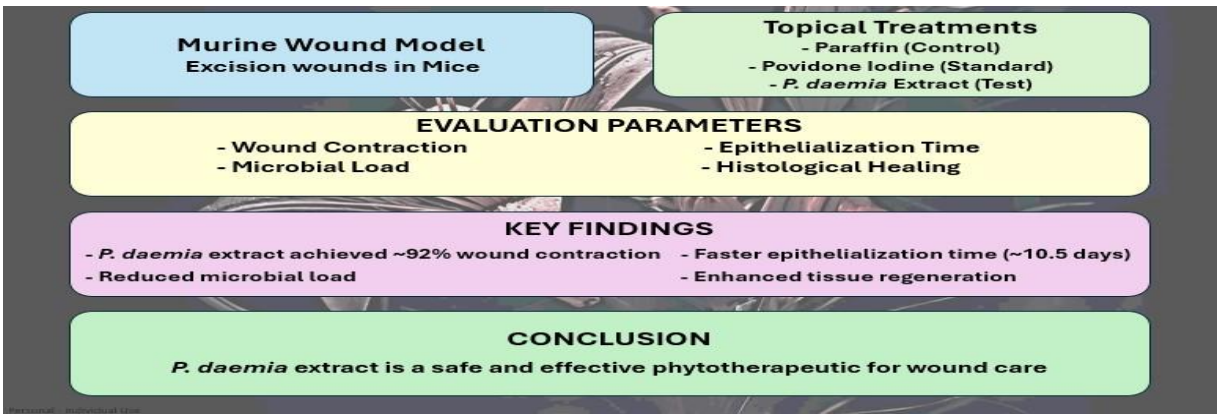
Abstract: *This research examines the wound healing efficacy of an ethanol extract derived from Pergularia daemia leaves, employing a full-thickness excision wound model in mice. The study contrasts the outcomes of the plant-based formulation with povidone-iodine (standard treatment) and paraffin (control). Animals were divided into three groups and treated topically for 14 consecutive days. Key healing parameters such as wound contraction rate, duration of epithelialization, microbial load reduction, and histopathological tissue response were assessed over a 14-day period. The findings indicate that the P. daemia extract demonstrates noteworthy wound healing and antimicrobial activity, nearly equivalent to the standard drug, underscoring its potential utility as a natural alternative in topical wound care therapies. These outcomes favor the medicinal value of P. daemia in topical wound care and encourage its integration into phytotherapeutic formulations as a cost-effective and natural alternative. Furthermore, its wide availability, historical ethnomedicinal usage, and biocompatibility make it a potential candidate for community-level therapeutic interventions where access to conventional medicine is limited. Additional studies focusing on molecular mechanisms and isolation of bioactive constituents will enhance our understanding of its mode of action and assist in developing novel botanical formulations.*

Keywords: *Pergularia daemia, wound repair, plant extract, mice model, povidone iodine, paraffin, phytomedicine, epithelial regeneration, microbial load*



INTRODUCTION:

The physiological repair of cutaneous injuries involves an orchestrated sequence of events comprising coagulation, inflammation, cellular proliferation, and tissue remodeling. Interruptions in this cascade can result in delayed or chronic wound states. With growing interest in botanical-based interventions, there is increasing scientific exploration into traditional herbs reputed for healing properties.

Belonging to the Asclepiadaceae family, Pergularia daemia is a twining herb widely recognized in indigenous medicine for managing dermatological conditions, infections, and inflammation. Its pharmacological potential is attributed to phytoconstituents like flavonoids, tannins, saponins, and alkaloids, which are known to contribute to tissue repair and antimicrobial action. Despite its traditional usage, a rigorous pharmacological evaluation of its wound healing and antimicrobial capacity is limited. This study aims to assess the reparative and antimicrobial effects of its ethanol leaf extract in vivo, juxtaposing it with paraffin and povidone iodine.

Moreover, the integration of herbal medicines in mainstream healthcare has received renewed attention globally due to concerns about resistance to synthetic antimicrobials and rising costs of allopathic wound care. Botanical agents like P. daemia not only offer antimicrobial support but may also activate endogenous repair mechanisms. In this context, examining the pharmacological profile and wound repair capabilities of such plants is essential to establishing their therapeutic roles based on scientific evidence.

MATERIALS AND METHODS:

2.1 Plant Source and Preparation of Plant Extract for Qualitative Phytochemical Screening

Mature Pergularia daemia leaves were harvested from a certified botanical garden and authenticated by a qualified taxonomist.

The collected leaves of Pergularia daemia were carefully washed with tap water to remove any adhering dust and impurities. The cleaned leaves were then air-dried under shade at ambient temperature to preserve phytoconstituents. Once dried, the material was coarsely ground using a mechanical grinder. The powdered sample was subjected to maceration in various solvents—methanol, ethanol, chloroform, and petroleum ether—for a duration of 3 to 5 days. In addition, an aqueous extract was also prepared by immersing the pulverized leaf material in distilled water. After the extraction period, all samples were filtered through Whatman No. 1 filter paper and stored in airtight containers for subsequent phytochemical analysis.

Preliminary phytochemical investigations were conducted following established protocols to identify the presence of major secondary metabolites.

Alkaloids (Mayer's Test)

To 1 mL of the extract, 1 mL of Mayer's reagent (KI solution) was added. The formation of a whitish yellow or cream coloured precipitate confirmed the presence of alkaloids.

Steroids (Libermann–Burchard Test)

A mixture of 2 mL acetic anhydride and 2 mL concentrated sulfuric acid was added to 1 mL of the extract. A colour change to violet, blue, or green indicated the presence of steroids.

Terpenoids (Salkowski Test)

To 1 mL of the extract, 2 mL of chloroform and few drops of conc. sulfuric acid were added. The formation of a reddish-brown ring at the interface suggested the presence of terpenoids.

Flavonoids (Alkaline Reagent Test)

A few drops of dilute ammonia solution followed by concentrated hydrochloric acid were added to 1 mL of the extract. The development of a yellow hue confirmed the presence of flavonoid compounds.

Saponins (Froth Test)

1 mL of the extract was mixed with 5 mL of distilled water and vigorously shaken. Sustained formation of froth was indicative of saponin content in the extract.

Phenols (Lead Acetate Test)

To 1 mL of the extract, 1 mL of lead acetate solution was added. The appearance of a precipitate confirmed the presence of phenolic compounds.

Tannins (Lead Acetate Test and Ferric Chloride Test)

1 mL of lead acetate was added to 1 mL of the extract. The appearance of a white precipitate was suggestive of tannin content in the sample

To 1 mL of the extract, 1 mL of $FeCl_3$ solution was added. A colour change to blue, black, or brownish green confirmed the presence of tannins.

Cardiac Glycosides (Keller–Killiani Test)

1 mL of the extract was mixed with 5 mL of distilled water and evaporated to dryness. The residue was treated with 2 mL of glacial acetic acid containing a trace of ferric chloride, followed by the careful addition of 1 mL concentrated sulfuric acid along the test tube wall. The presence of cardiac glycosides was confirmed by the formation of a brown ring underlying a bluish layer.

Amino Acids (Ninhydrin Test)

To 1 mL of the extract, 3–4 drops of ninhydrin solution were added and the mixture was heated in a water bath for 10 minutes. The development of a purple or blue colour indicated the presence of amino acids.

Proteins (Biuret Test)

1 mL of the extract was treated with 1 mL of 40% sodium hydroxide and 2 drops of 1% copper sulfate solution. A violet coloration confirmed the presence of proteins.

Carbohydrates (Barfoed's Test)

To 2 mL of the extract, 1 mL of Barfoed's reagent was added and the mixture was heated in a water bath. The formation of a reddish-brown precipitate indicated the presence of carbohydrates.

Reducing Sugars (Fehling's Test)

Equal volumes of Fehling's solution A and B were added to 1 mL of the extract and the mixture was heated. The appearance of a brick-red precipitate confirmed the presence of reducing sugars.

2.2 Preparation of Plant Extract for Pharmacological Evaluation

The plant material was shade-dried, milled, and subjected to Soxhlet extraction using ethanol (90%). The concentrated extract was stored under refrigeration and incorporated into a 5% (w/w) ointment base for topical application.

The choice of ethanol as the solvent was guided by its efficiency in extracting polar phytochemicals, which include bioactive compounds like phenolics and flavonoids. Following evaporation, the crude extract was semi-solid and stored in sterile glass containers under refrigeration (4°C) to preserve stability until formulation. A paraffin-based ointment base was selected due to its inert nature and wide compatibility with various plant-derived actives.

2.3 Experimental Animals and Ethics

Swiss albino mice (25–30g) were acclimatized under standard laboratory conditions. The study adhered to the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and received approval from the Institutional Animal Ethics Committee (IAEC No.: 1877/PO/Re/S/16/CCSEA/2023/007).

Prior to the experiment, all mice had undergone a week of acclimatization period. Bedding was changed regularly, and animals were monitored daily for general health, signs of stress, and any unexpected behavioral changes. Randomization was applied to minimize bias in group allocation. All efforts were made to reduce the number of animals used while ensuring statistical power.

2.4 Wound Induction and Treatment Groups

A full-thickness circular excision wound (~300 mm2) was surgically induced on the dorsal surface under ketamine (50 mg/kg) and xylazine (10 mg/kg) anesthesia. Animals were randomized into three groups (n=6 each):

Group I (Control): Treated with paraffin base only

Group II (Standard): Treated with povidone iodine ointment

Group III (Test): Treated with 5% P. daemia extract ointment

Each formulation was applied topically once daily for 14 days. Wound area was traced on transparency sheets and measured on Days 0, 4, 8, and 14 using graph paper.

Animals were closely monitored postoperatively to ensure there were no signs of distress or wound interference. All wounds were left open, and dressing was avoided to simulate normal wound exposure. Efforts were taken to prevent self-mutilation and cross-contamination between animals. Group allocation and daily treatments were performed in a blinded manner.

2.5 Assessment Parameters

Wound Contraction Dynamics:

Wound Contraction (%) calculated as: Percentage Wound Contraction = [(Initial area – Day X area) / Initial area] × 100

Microbial Load: Sterile swabs collected from wounds on Days 4, 8, and 14. Cultured on nutrient, MacConkey, and Sabouraud dextrose agar; CFUs counted after 24–48 h incubation at 37°C.

In addition to the above, general behavior, food intake, and weight changes were monitored throughout the study to assess any systemic effects of the formulations. Scoring criteria for histopathology included granulation tissue formation, epithelial continuity, and inflammatory cell density. These semi-quantitative scores were evaluated by two independent pathologists blinded to group identity.

2.6 Statistical Interpretation

Data are presented as mean ± SEM. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test, with a significance threshold of $p < 0.05$.

RESULTS

3.1 Qualitative assessment of bioactive constituents in the leaves of Pergularia daemia

A summary of the qualitative phytochemical profiling of Pergularia daemia leaf extracts is presented in Table 1. Both methanolic and ethanolic extracts revealed a rich diversity of bioactive compounds, including alkaloids, steroids, flavonoids, terpenoids, saponins, phenols, tannins, cardiac glycosides, amino-acids, proteins, carbohydrates, and reducing sugars.

Table 1. Qualitative assessment of bioactive constituents

Tests	Methanol	Ethanol	Petroleum Ether	Chloroform	Aqueous
Alkaloid	+	+	-	-	+

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Steroids	+	+	+	+	+
Flavonoids	-	+	+	-	+
Terpenoids	+	+	+	-	+
Saponins	+	+	+	+	+
Phenols	+	+	-	+	-
Tannins	+	+	+	-	+
Cardiac Glycosides	+	+	+	+	+
Amino acids	+	-	-	-	-
Proteins	+	-	+	-	-
Carbohydrates	+	-	-	-	-
Reducing Sugar	+	+	+	+	+

3.2 Wound Contraction Dynamics:

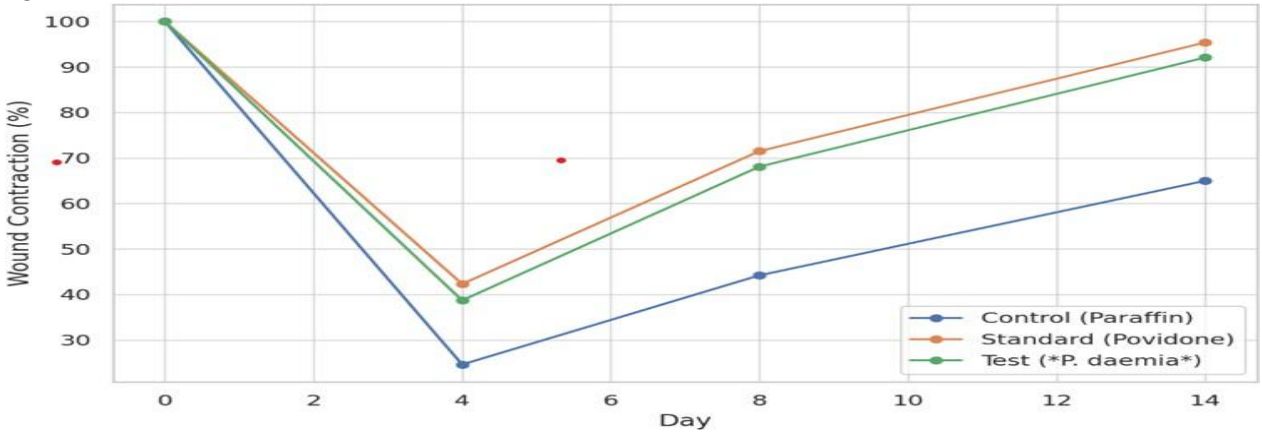
All groups showed progressive wound contraction over time. By Day 14, the test group treated with P. daemia showed 92.1% contraction, which was close to the 95.4% observed in the standard group. The control group showed only 65.0%, indicating a slower healing rate (Table 2).

This pattern indicates that P. daemia facilitated wound closure at a rate similar to povidone-iodine, suggesting biological activity that promotes tissue remodeling. The largest increase in contraction occurred between Day 4 and Day 8 in both the test and standard groups. These differences were statistically significant ($p < 0.05$), establishing the extract's influence during the proliferative phase of healing.

Table 2. Time-course data on wound contraction

Time Point (Day)	Control Group (Paraffin)	Standard Group (Povidone)	Test Group (P. daemia)
0	100%	100%	100%
4	24.6 ± 2.1	42.3 ± 2.8	38.7 ± 3.0
8	44.2 ± 3.5	71.5 ± 2.4	68.1 ± 2.9
14	65.0 ± 4.3	95.4 ± 1.2	92.1 ± 1.7

Figure 1. Wound Contraction Over Time



3.3 Microbial Load (CFU/mL):

CFU counts showed a significant reduction in microbial load in the test and standard groups over time. On Day 14, microbial presence was nearly undetectable in these groups, while control samples retained notable microbial counts (Table 3).

The decline in both bacterial and fungal CFUs indicates that the P. daemia extract possesses broad-spectrum antimicrobial properties. By Day 8, both the standard and test formulations had substantially reduced pathogen counts, supporting their antiseptic effectiveness. Notably, Gram-negative bacteria showed greater sensitivity to the treatments, aligning with the known vulnerability of these organisms to membrane-active phytoconstituents.

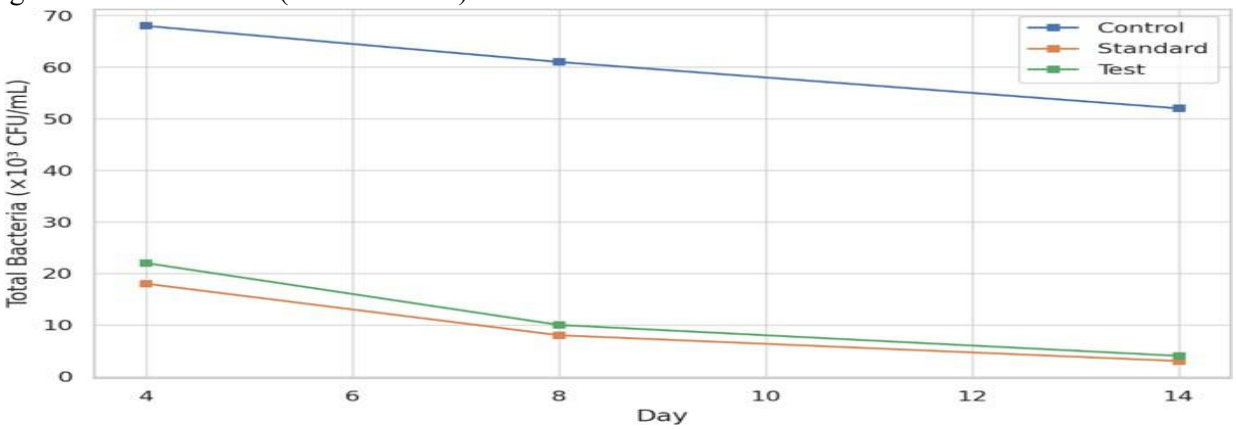
Table 3. Microbial load quantification

Day	Treatment Group	Total Bacteria (×103 CFU/mL)	Gram-negative (×103 CFU/mL)	Fungi (×10² CFU/mL)
4	Control	68 ± 4.5	45 ± 3.2	21 ± 1.8

4	Standard (Povidone)	18 ± 2.0	10 ± 1.1	3 ± 0.5
4	Test (P. daemia)	22 ± 2.4	12 ± 1.3	4 ± 0.6
8	Control	61 ± 3.9	42 ± 2.9	17 ± 1.4
8	Standard	8 ± 0.8	4 ± 0.6	1 ± 0.2
8	Test	10 ± 1.1	5 ± 0.7	1 ± 0.2
14	Control	52 ± 3.5	34 ± 2.6	14 ± 1.3
14	Standard	3 ± 0.5	1 ± 0.2	ND
14	Test	4 ± 0.6	1 ± 0.3	ND

ND = Not Detectable

Figure 2. Microbial Load (Total Bacterial)



DISCUSSION

Wound repair can be enhanced by agents that exhibit anti-inflammatory, antioxidant, antimicrobial, and proliferative effects. The performance of P. daemia extract in this study—marked by faster wound contraction, reduced microbial colonization, and epithelial coverage—likely stems from its bioactive constituents such as flavonoids, tannins, and saponins. These compounds are known to promote fibroblast proliferation, collagen synthesis, capillary formation, and antimicrobial action.

Histological evidence supports these findings, showing restored epidermal layers and reduced leukocytic infiltration in the test group. The microbial assay indicates a significant reduction in bacterial and fungal load, paralleling povidone's antiseptic profile. These outcomes validate the ethnopharmacological relevance of P. daemia in wound management.

Future research should focus on isolating specific active principles, understanding their molecular mechanisms, and conducting dose-response assessments to facilitate clinical translation.

Furthermore, the inclusion of P. daemia in wound care may offer an eco-friendly and sustainable therapeutic avenue, especially for rural and low-income communities. Its dual pharmacodynamic actions—wound contraction and antimicrobial defense—provide a strategic advantage over single-mechanism agents. Expanding this line of research with comparative clinical trials and detailed phytochemical analyses will clarify its role in integrative medicine.

CONCLUSION

The topical application of Pergularia daemia ethanolic leaf extract significantly enhances wound healing and reduces microbial burden in a murine excision model. Its efficacy, comparable to that of povidone iodine, supports its potential integration into phytotherapeutic wound care formulations.

These findings add to the growing evidence base supporting traditional medicinal plants in modern therapeutics. With its demonstrated safety and effectiveness in a controlled experimental setup, P. daemia may serve as a low-cost, accessible option in community healthcare settings. Integration with current topical formulations and further pharmacological validation could advance its clinical relevance.

Additionally, the dual wound healing and antimicrobial action of the extract suggests a multifunctional benefit, making it particularly suitable for use in environments with limited access to synthetic antiseptics. The observed histopathological improvements highlight its potential to accelerate tissue regeneration and reduce

complications like secondary infections. The data obtained encourage broader pharmacognostic exploration of Asclepiadaceae family plants and support further in vivo and clinical validation.

In addition to the above findings, the study contributes to the growing body of evidence supporting traditional medicinal knowledge with modern pharmacological validation. By scientifically demonstrating the therapeutic benefits of Pergularia daemia in wound repair, this research reinforces the potential for integrating herbal remedies into primary healthcare frameworks. Such integration is especially crucial in resource-constrained regions where access to synthetic drugs is limited or cost-prohibitive.

Furthermore, the extract's effectiveness in both promoting tissue regeneration and controlling microbial populations positions it as a dual-action agent, reducing the need for separate antimicrobial and healing products. This multifunctional role may lower treatment complexity, improve patient compliance, and reduce overall healthcare costs. From a formulation science perspective, the compatibility of the extract with a simple ointment base also offers opportunities for scale-up and commercialization.

Future directions should explore advanced drug delivery systems for the extract, such as hydrogels, nano emulsions, or biodegradable scaffolds, to enhance bioavailability and therapeutic outcomes. Additionally, investigating synergistic effects with other herbal or synthetic agents may yield novel combination therapies with broader efficacy.

Collectively, the outcomes of this study lay a strong groundwork for translational research, moving from bench to bedside. With continued investigation, Pergularia daemia could emerge as a standardized botanical therapeutic in modern wound care protocols, fulfilling both clinical efficacy and environmental sustainability goals.

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Conflicts of Interest: The authors declare no conflicts of interest related to this study.

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Figures & Tables:

Table 1. Qualitative assessment of bioactive constituents.
Table 2. Time-course data on wound contraction.
Table 3. Microbial load quantification.
Figure 1. Wound Contraction Over Time.
Figure 2. Microbial Load (Total Bacterial).

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