Pharmacogn. Res., 2027; 19(2):673-681.
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Original Article

Exploring the Therapeutic Potential of *Ficus benghalensis* Bark: A Comparative Study of Aqueous and Ethanolic Extracts in Wound Healing

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ABSTRACT

Introduction: Wound healing is a complex and coordinated biological process involving inflammation, proliferation, tissue formation, and remodelling. The present study aimed to evaluate and compare the wound-healing potential of aqueous and ethanolic extracts of *Ficus benghalensis* bark using experimental animal models. **Materials and Methods:** The wound-healing activity of the extracts was evaluated using incision and excision wound models in albino rats. In the incision model, the breaking strength of the repaired skin was assessed as the primary parameter. In the excision model, percentage wound contraction and epithelialization period were evaluated. Povidone-iodine cream was used as the standard treatment for comparison. **Results:** Both aqueous and ethanolic extracts demonstrated significant wound-healing activity. However, the aqueous extract showed comparatively greater efficacy than the ethanolic extract and control groups, as evidenced by a greater increase in breaking strength in the incision model, enhanced wound contraction, and a shorter epithelialization period in the excision model. **Conclusion:** The findings demonstrate that the aqueous extract of *Ficus benghalensis* bark possesses greater wound-healing potential than the ethanolic extract. The results suggest that *F. benghalensis* aqueous extract may serve as a promising natural therapeutic candidate for wound management and warrants further investigation.

Keywords: Excision, *Ficus benghalensis*, Incision, Phytochemical analysis, Povidone-iodine cream.

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Received: 21-08-2026;
Revised: 11-09-2026;
Accepted: 08-10-2026.

INTRODUCTION

Inflammation, cell proliferation, and tissue regeneration are three ordered but temporally overlapping steps of the wound repair process (Nagar *et al*., 2016). The process of wound healing is essentially a response of connective tissue. It begins with an acute inflammatory phase, followed by the production of collagen and other extracellular macromolecules, which are subsequently remodeled to form scar tissue. Thus, wound healing and tissue restoration are intricate processes involving a number of cellular and molecular interactions. Numerous publications have reported that plant extracts possess wound-healing properties (Dewangan *et al*., 2012; Kokane *et al*., 2009; Nagar *et al*., 2016).

Scientific investigations have demonstrated the prohealing properties of several medicinal plants. *Aloe vera, Leucas lavandulifolia, Trigonella foenum-graecum*, and *Tridax procumbens* have all demonstrated encouraging wound-healing properties.

The *Moraceae* family member *Ficus benghalensis* Linn. is an evergreen, moderately to large-sized, spreading, lactiferous tree with noticeable aerial barks that grows in various regions of India. It is frequently grown in villages and is also valued for its medicinal properties. The bark possesses astringent properties and has traditionally been used for conditions such as haemoptysis, menorrhagia, and dysentery, as well as a mouthwash for spongy gums. In the traditional Indian system of medicine, various parts of the plant, including the leaves, fruits, bark, latex, and barks, have been used for therapeutic purposes (Patil and Patil, 2010).

The bark has been reported to possess antibacterial, antipyretic, and vermicidal properties, while its decoction has traditionally been used for the management of diabetes, ulcers, and certain skin disorders. The plant has also been traditionally used for piles, dysentery, asthma, gonorrhea, leucorrhea, and urinary disorders, and as a poultice for inflammatory swellings and boils (Patil and Patil, 2010). *F. benghalensis* has been investigated for several phytochemical constituents, including campesterol, hentriacontane, hentriacontanol, kaempferol, stigmasterol, methyl ellagic acid, glauanol acetate, racemosic acid, lupeol

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DOI: 10.5530/pres.20270230

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acetate, friedelin, cycloartenol, euphorbol and its hexacosanoate, taraxerone, and other phytosterols (Patil and Patil, 2010; Subramanian and Misra, 1978).

The wound-healing potential of the bark of *F. benghalensis* has not been adequately investigated. Therefore, the present study was undertaken to evaluate and compare the wound-healing activity of aqueous and ethanolic bark extracts of *F. benghalensis* using experimental animal models (Figure 1).

Preliminary Phytochemical Analysis

Preliminary phytochemical screening was carried out to identify the major classes of phytoconstituents present in the ethanolic and aqueous bark extracts of *Ficus benghalensis*. The extracts were screened for the presence of various phytochemical groups using standard pharmacognostical and phytochemical procedures (Kokate, 1986).

Column Chromatography of the Bioactive Ethanolic Extract

Thin-Layer Chromatographic (TLC) evaluation of the selected bioactive extract was initially performed to assess the complexity of the phytochemical constituents and to facilitate their separation. The TLC profile indicated the presence of multiple phytoconstituents; therefore, column chromatography was employed for further separation and fractionation of the bioactive ethanolic extract.

Procedure

Activated silica gel was mixed with *n*-hexane to prepare a slurry. The slurry was carefully poured into a glass chromatography column that had been previously equilibrated with the solvent.

A small quantity of cotton was placed at the bottom of the column to provide support and ensure uniform packing of the adsorbent. The silica gel slurry was then allowed to settle uniformly before sample loading.

The bioactive ethanolic extract was mixed thoroughly with silica gel until a free-flowing, uniformly adsorbed sample was obtained.

The prepared sample was carefully loaded onto the packed silica gel column. A thin layer of cotton was placed over the loaded sample to prevent disturbance of the sample bed during addition of the mobile phase. The solvent level was maintained above the sample throughout the separation process.

The column was subsequently eluted using an appropriate mobile-phase system in a graded manner. Fractions were collected separately, and a selected fraction (100 mL) was collected and concentrated at room temperature to obtain a solid fraction for further analysis.

Thin-Layer Chromatography of Bioactive Fractions

The bioactive fractions obtained from column chromatography were subjected to Thin-Layer Chromatography (TLC) for preliminary profiling and identification of the phytochemical constituents present in the fractions. The TLC profiles were compared based on the number, position, and appearance of the spots obtained after development.

Experimental Animals And Ethical Approval

Wistar albino rats weighing 180-200 g were used for the wound-healing experiments. The animals were procured from Jai Foundation Research, Vapi, Gujarat, India. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Invitox R and D Institute, Pune, under project proposal number IRDI/IAEC/M05/14/2025-26, entitled "Formulation and Pharmacological Evaluation of Poly Herbal Gel for Wound Healing Activity." The proposal was approved during the IAEC meeting held on 05 May 2025, and 72 Wistar rats were sanctioned under the approved protocol for the excision wound model for a period of one year.

All experimental procedures involving animals were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Prior to experimentation, the animals were acclimatized for one week under standard laboratory conditions. They were maintained at ambient temperature with a 12-hr light/dark cycle and relative humidity of 60±5%. The animals were provided with a standard pellet diet and water *ad libitum* throughout the experimental period. The wound-healing activity was evaluated following topical application of the aqueous and ethanolic bark extracts of *Ficus benghalensis*.

Incision Wound Model

The incision wound model was used to evaluate the effect of the test extracts on wound breaking strength. The rats were anesthetized using ketamine (0.5 mL/kg body weight, intraperitoneally). Approximately 30 min after administration of ketamine, the dorsal surface of each rat was shaved and disinfected. A longitudinal incision approximately 6 cm in length and 2 mm in depth was made on the shaved dorsal skin using a sterile scalpel.

The divided skin edges were carefully approximated and sutured at approximately 0.5 cm intervals using black silk surgical thread (No. 000) with a curved needle (No. 9). The sutures were tightened adequately to ensure proper closure of the wound. The respective treatments were applied topically to the wounds once daily for 10 consecutive days.

The animals were divided into four groups, with six rats in each group. Group I received simple cream and served as the control

group. Group II received povidone-iodine cream and served as the standard treatment group. Groups III and IV received the ethanolic and aqueous bark extracts of *Ficus benghalensis*, respectively.

Day 0 was considered the day of wound induction. The sutures were removed on the eighth post-wounding day. On the tenth day, the tensile strength of the healed skin was determined using a tensiometer. Breaking strength was expressed as the weight in grams required to rupture the healed wound. Increased breaking strength was considered indicative of improved wound healing (Nagar *et al.*, 2016; Kokane *et al.*, 2009).

Excision Wound Model

The excision wound model was employed to evaluate wound contraction and epithelialization. A circular template was used to produce a uniform excision wound of approximately 2 cm in diameter on the shaved dorsal surface of the rats. The wounds were treated according to the respective treatment groups described above.

The wound area was measured at predetermined intervals until complete epithelialization. The percentage of wound contraction was calculated from the changes in wound area relative to the initial wound area. The period of epithelialization was recorded as the number of days required for complete disappearance of the raw wound surface and formation of new epithelium. The scar area after complete epithelialization was also recorded. The wound-healing activity of plant extracts has previously been evaluated using similar experimental wound models (Garg and Paliwal, 2011; Kokane *et al.*, 2009).

Histopathological Examination

For histopathological examination, skin tissue specimens were collected from the healed wound areas of the control, standard, and treatment groups following completion of the wound-healing experiments. The tissue samples were processed, sectioned, and stained with Hematoxylin and Eosin (H&E). The stained sections were examined under a light microscope for histological changes, including collagen deposition, fibroblast proliferation, inflammatory cell infiltration, re-epithelialization, and tissue organization. Histopathological examination was used to support the macroscopic findings of wound healing (Nagar *et al.*, 2016; Ergene Öz *et al.*, 2017).

Statistical Analysis

All experimental data were expressed as Mean±Standard Error of the Mean (SEM). Statistical analysis was performed using one-way Analysis of Variance (ANOVA), followed by Dunnett's *post hoc* test for comparison between the treatment groups and the control group. A value of $p<0.05$ was considered statistically significant.

RESULTS

Preliminary Phytochemical Analysis

Preliminary phytochemical screening of the bark extracts of *Ficus benghalensis* revealed the presence of saponins, tannins, alkaloids, and flavonoids, whereas other phytochemical constituents, including amino acids and carbohydrates, were not detected under the conditions employed. The phytochemical profile was further supported by chromatographic fingerprinting. These findings are consistent with previous reports describing the presence of diverse phytoconstituents in *Ficus benghalensis* (Patil and Patil, 2010; Subramanian and Misra, 1978).

Incision Wound Model

The drug-treated groups exhibited a significant increase in the breaking strength of the healed incision wounds compared with the control group ($p<0.05$). The breaking strength increased from 257.50±2.81 g in the control group to 497.20±3.03 g in the group treated with the aqueous bark extract of *Ficus benghalensis*. The ethanolic bark extract-treated group showed a breaking strength of 394.70±6.61 g, whereas the povidone-iodine-treated standard group showed a breaking strength of 352.00±2.83 g.

Among the treatment groups, the aqueous bark extract produced the highest breaking strength, indicating enhanced wound repair and increased tensile strength of the healed tissue. The differences between the extract-treated groups and the control group were statistically significant ($p<0.05$). The results of the

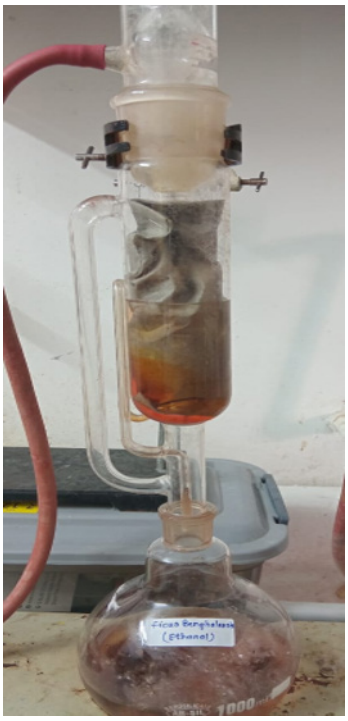


Figure 1: Extraction process using ethanol.

breaking-strength assessment in the incision wound model are presented in Figure 2.

Control St. Povidone-iodine cream Aq. Extract of F.B Ethanolic of F.B

Excision Wound Model

The effect of the aqueous and ethanolic bark extracts of *Ficus benghalensis* on wound healing was evaluated using the excision wound model. The changes in wound area at different observation periods are presented in Table 1.

The control group showed minimal reduction in wound area during the experimental period, with the wound area decreasing from 334.58±12.25 at baseline to 326.04±89.23 on the 14th day, corresponding to approximately 2.55% wound contraction. In contrast, the standard treatment group showed a progressive reduction in wound area from 396.19±43.56 at baseline to 224.73±78.63 on the 14th day, corresponding to 43.28% wound contraction.

The ethanolic bark extract-treated group demonstrated a progressive reduction in wound area from 365.07±56.25 at baseline to 208.05±45.36 on the 14th day (Table 2). The corresponding percentage wound contraction was 2.08%, 23.68%, and 43.01% on the 1st, 7th, and 14th days, respectively.

The aqueous bark extract-treated group showed a reduction in wound area from 311.65±45.32 at baseline to 166.41±41.23 on the 14th day. The percentage wound contraction was −1.56%, 14.17%, and 46.60% on the 1st, 7th, and 14th days, respectively. On the 14th day, the aqueous extract-treated group showed the highest percentage of wound contraction (46.60%) among all groups, indicating a comparatively better wound-healing response.

Table 1: Effect of aqueous and ethanolic bark extracts of *Ficus benghalensis* on wound area and percentage wound contraction in the excision wound model.

Group	Treatment	Initial wound area (Day 0)	Day 1	Day 7	Day 14
Group I (F1)	Control (simple cream)	334.58±12.25	341.58±12.39	330.54±54.36	326.04±89.23
Group II (F2)	Standard (povidone-iodine cream)	396.19±43.56	388.27±78.25	299.10±54.21	224.73±78.63
Group III (F3)	Ethanolic bark extract (200 mg/kg)	365.07±56.25	357.48±38.69	278.63±45.21	208.05±45.36
Group IV (F4)	Aqueous bark extract (200 mg/kg)	311.65±45.32	316.51±46.25	267.50±78.32	166.41±41.23

Values are expressed as *mean±SEM* (*n*=3).

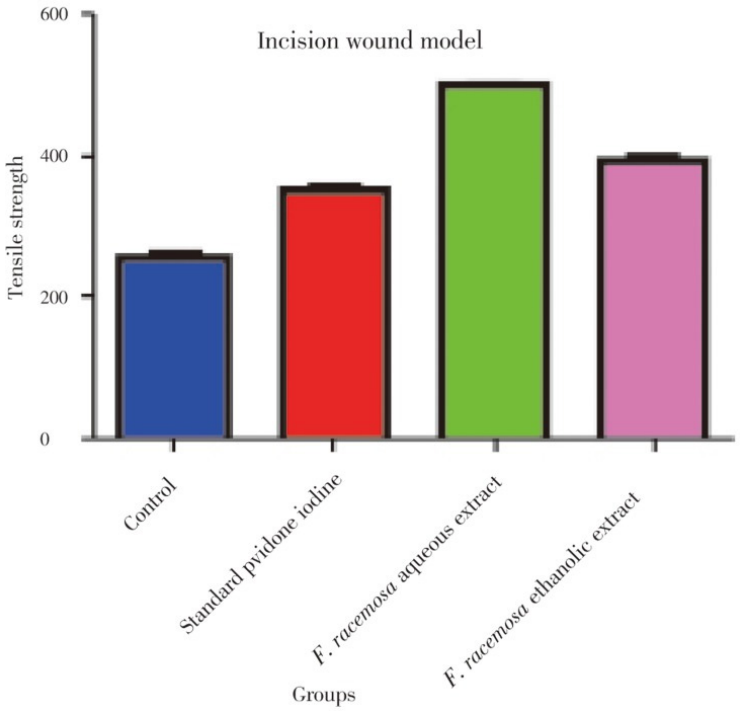


Figure 2: Measurement of breaking strength in incision model.

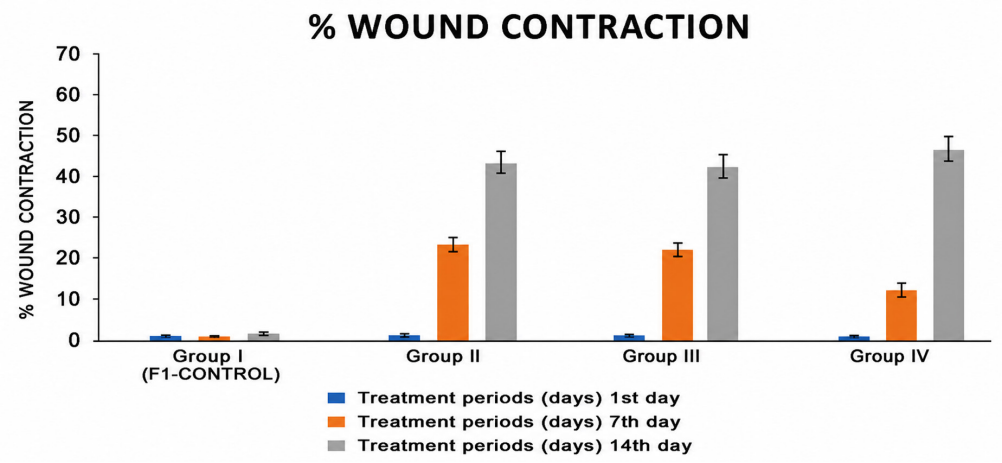


Figure 3: % Wound Contraction.

Overall, both extracts demonstrated a greater reduction in wound area than the control group. The aqueous bark extract showed the highest percentage wound contraction on the 14th day, followed closely by the standard treatment and ethanolic extract groups (Figure 3) (Garg and Paliwal, 2011).

Histopathological Analysis

Histopathological examination was performed to further evaluate the tissue-level changes associated with wound healing in the control, standard, and extract-treated groups. The histological sections were examined for inflammatory cell infiltration, fibroblast proliferation, collagen deposition, re-epithelialization, and overall tissue organization. The observed histopathological changes supported the macroscopic findings of enhanced wound healing in the extract-treated groups, particularly in the group treated with the aqueous bark extract of *Ficus benghalensis*.

In vivo wound healing activity: Excision model

Excision and incision-type wounds in albino rats treated with normal medication and *F. benghalensis* extracts had considerable healing, as seen in fibroblast cells, collagen fibers, and new blood vessels in Figures 4 and 5, whereas wounds in control rats exhibited inadequate healing in Figure 6. When compared to the groups treated with extracts and reference Cream, the control group shown the least capacity for wound healing. When compared to the control group, fibroblast cells, collagen fibers, and blood vessels are more prevalent in the standard and extract-treated groups (Figure 7).

DISCUSSION

Haemostasis, inflammation, proliferation, and remodelling or maturation constitutes the sequential and overlapping phases of wound healing. These phases involve a coordinated series of cellular and molecular events that regulate tissue defence, repair, re-epithelialization, collagen deposition, angiogenesis, and tissue remodelling (Ergene Öz *et al.*, 2017; Nagar *et al.*, 2016). The wound-healing response is influenced by complex biological mechanisms that regulate the body's cellular defence and tissue repair processes. Thus, the healing potential of *Ficus benghalensis* aqueous and ethanolic extracts on different phases of wound healing was evaluated in the present study using two distinct experimental wound models (Figure 8).

Increased collagen concentration and improved stability and organization of collagen fibres may be responsible for the increased tensile strength of the treated incision wounds. Collagen, which is predominantly synthesized by fibroblasts during the proliferative phase, plays a critical role in the development and strengthening of newly formed tissue (Kokane *et al.*, 2009; Nagar *et al.*, 2016). Antioxidant activity and enhanced blood vessel formation may also contribute to improved tissue repair by reducing oxidative stress and promoting adequate vascularization at the wound site (Ergene Öz *et al.*, 2017). The findings of the present study indicate that *F. benghalensis* possesses considerable wound-healing potential.

The groups treated with aqueous and ethanolic extracts showed an increase in the breaking strength of the incision wounds. Increased breaking strength is an important indicator of improved collagen deposition and maturation of the repaired tissue. Similar wound-healing activity of aqueous and ethanolic extracts of *Ficus benghalensis* has been previously reported, supporting the findings of the present investigation (Garg and Paliwal, 2011).

The aqueous and ethanolic extracts of the *F. benghalensis* bark significantly increased the percentage of wound closure in the excision wound-healing model, which may be associated with improved epithelialization. The effect of *F. benghalensis* extracts on collagen synthesis and tissue regeneration may contribute to the observed increase in epithelialization and wound contraction. A higher percentage of wound closure and increased breaking strength are considered indicators of improved wound-healing activity (Garg and Paliwal, 2011; Kokane *et al.*, 2009). Thus, the

Table 2: Effect of different treatments on percentage wound contraction in the excision wound model.

Groups (*n*=6)	Treatment	1st day (%)	7th day (%)	14th day (%)
Group I (F1)	Control (simple cream)	2.09±0.36	1.20±0.23	2.55±0.12
Group II (F2)	Standard drug (povidone-iodine cream)	1.99±0.19	24.50±0.12	43.27±0.98
Group III (F3)	Ethanolic bark extract (100 mg/kg)	2.07±0.48	23.67±0.28	43.01±0.85
Group IV (F4)	Aqueous bark extract (200 mg/kg)	1.55±0.98	14.16±0.26	46.60±0.57

Values are expressed as mean±SEM (*n*=6). *:*p*<0.05 when compared with control group.

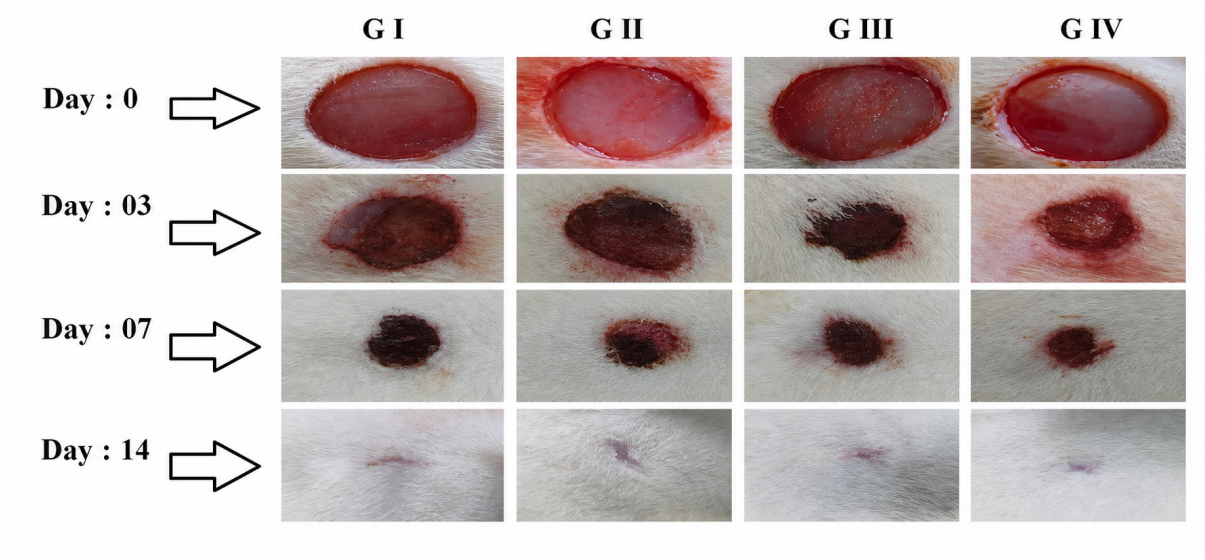


Figure 4: Wound Healing Activity Wistar Rat.

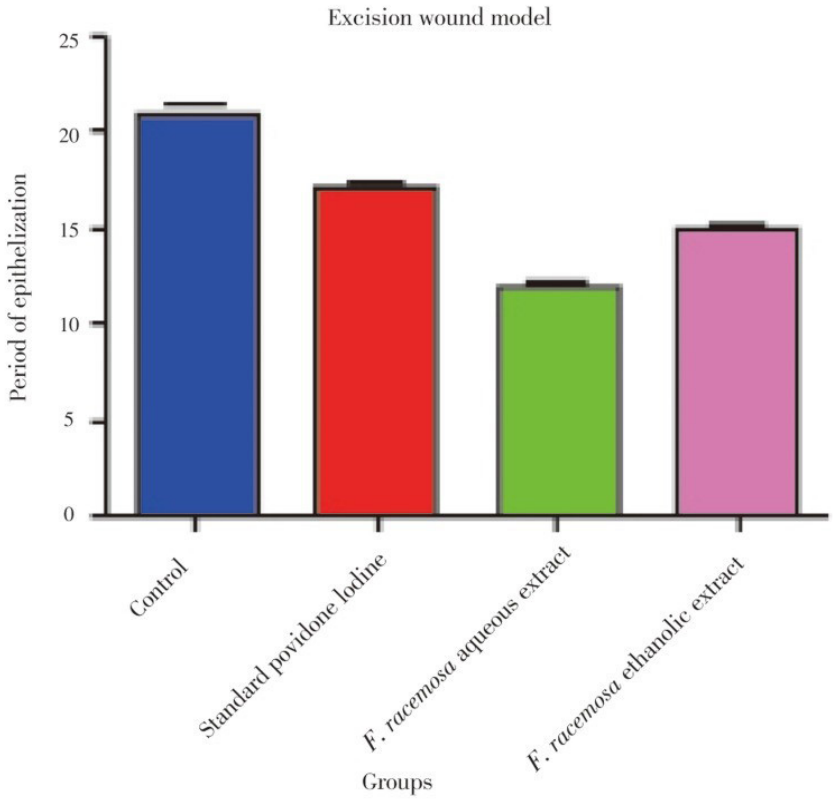


Figure 5: Measurement of period of epithelialization in excision model. Control Std. povidone iodine cream aq. Extract of F.B Ethanolic of F.B.

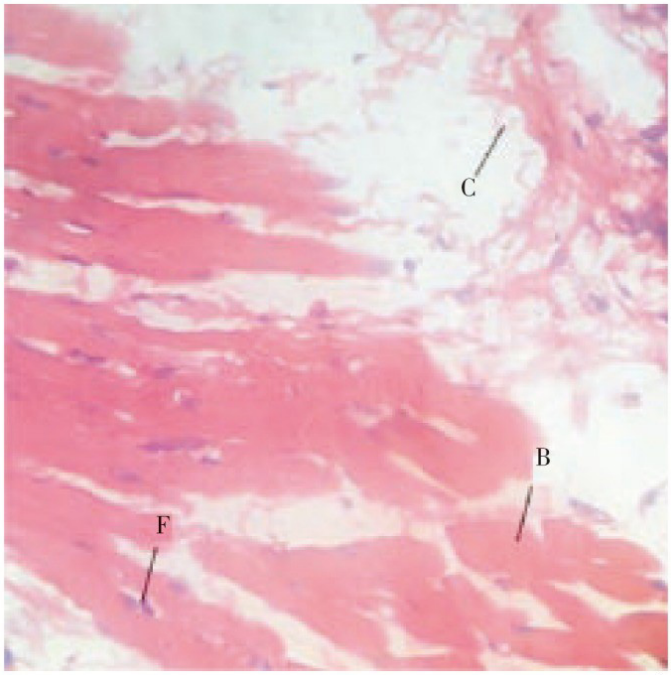


Figure 6: Histopathological characteristics of healed tissue on the 10th day by treatment with standard Cream. The figure shows increased fibroblasts cells (F), collagen fibres (C) and blood vessels (B) in incision wound.

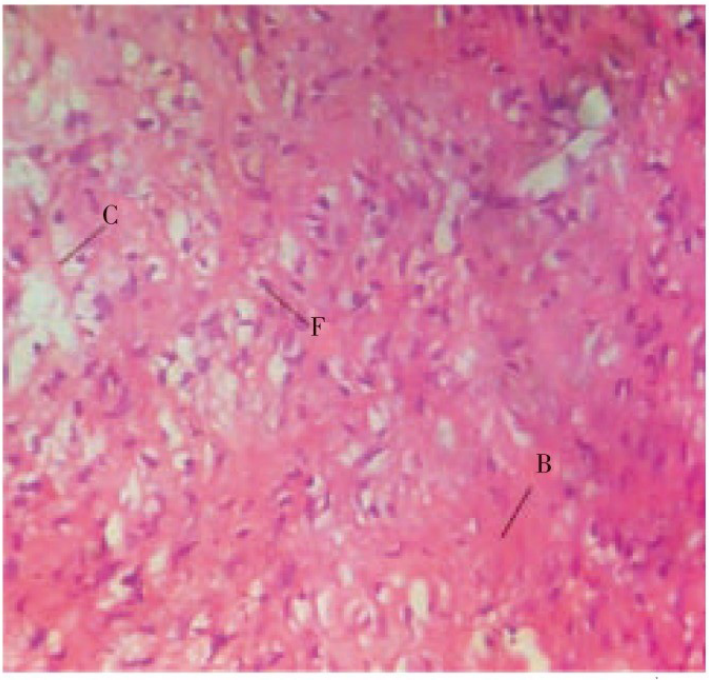


Figure 7: Histopathological characteristics of rat skin on the 10th day by treatment with*F. benghalensis* extracts. The figure shows increased fibroblasts cells (F), collagen fibres (C) and blood vessels (B) in incision wound.

present findings further support the wound-healing potential of *F. benghalensis*.

Phytochemical constituents such as flavonoids, alkaloids, saponins, tannins, and other phenolic constituents have been reported to contribute to the wound-healing properties of medicinal plants through antioxidant, anti-inflammatory, antimicrobial, and tissue-regenerative activities (Dewangan *et al.*, 2012; Kokate, 1986). Phytochemical studies and pharmacological investigations of *F. benghalensis* have also reported the presence of several bioactive constituents that may contribute to its therapeutic effects (Patil and Patil, 2010; Subramanian and Misra, 1978). Therefore, the wound-healing activity observed in both the aqueous- and ethanolic-extract-treated groups could be attributed

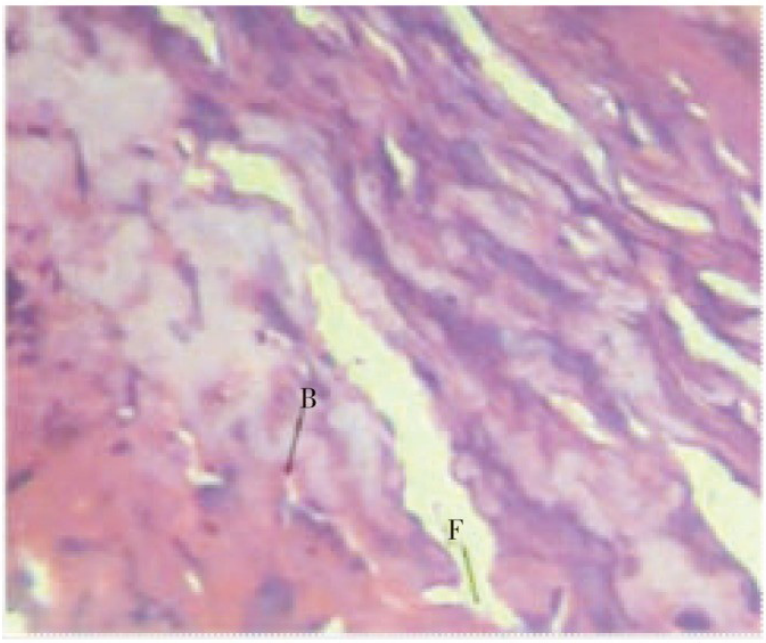


Figure 8: Histopathological characteristics of rat skin on the 10th day by treatment with Cream base. The figure shows poor fibroblast cells (F) and blood vessels (B) in incision wound.

to the individual or synergistic actions of phytoconstituents such as flavonoids, alkaloids, saponins, tannins, and other bioactive compounds.

CONCLUSION

The present study demonstrated that both aqueous and ethanolic bark extracts of *Ficus benghalensis* possess promising wound-healing activity in incision and excision wound models. However, the aqueous extract exhibited superior wound-healing efficacy, as evidenced by greater breaking strength, enhanced wound contraction, and a shorter epithelialization period compared with the ethanolic extract. These findings suggest that the aqueous bark extract of *Ficus benghalensis* may serve as a promising natural source for wound management. Further studies are warranted to identify the active phytoconstituents and elucidate the underlying mechanisms responsible for its wound-healing activity.

ACKNOWLEDGEMENT

The authors are thankful **Dr. Vinod B.:** Shimpale (M.Sc., Ph.D., FIAAT) Professor at The New College, Kolhapur, for plant identification and authentication. The authors also express their sincere gratitude to the Department of Pharmaceutics, **ASPM's K. T.:** Patil College of Pharmacy, for providing the necessary research facilities to carry out this work.

ABBREVIATIONS

ANOVA: Analysis of Variance; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **HPTLC:** High-Performance Thin-Layer Chromatography; **IAEC:** Institutional Animal Ethics Committee; **SEM:** Standard Error of the Mean; **TLC:** Thin-Layer Chromatography.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

A. Conceptualization: Swati Siddheshwar Londhe, Amir I. Quazi and Mangesh Gautam Bhosale. **B. Methodology:** Swati Siddheshwar Londhe and Mangesh Gautam Bhosale. **C. Investigation and Data Collection:** Swati Siddheshwar Londhe and Mangesh Gautam Bhosale. **D. Data Analysis and Interpretation:** Swati Siddheshwar Londhe and Mangesh Gautam Bhosale. **E. Supervision and Project Administration:** Amir I. Quazi. **F. Writing – Original Draft:** Swati Siddheshwar Londhe and Mangesh Gautam Bhosale. **G. Writing – Review, Editing, and Major Revision:** Swati Siddheshwar Londhe and Mangesh Gautam Bhosale, with guidance from Amir I. Quazi. **H. Final Approval and Accountability:** All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

ETHICAL APPROVAL

The experimental animal study was conducted in accordance with the relevant institutional guidelines for the care and use of laboratory animals. Ethical approval was obtained from the Institutional Animal Ethics Committee (IAEC) under approval number **IRDI/IAEC/M05/14/2025-26.** All experimental procedures involving animals were performed in accordance with the approved protocol.

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Cite this article: Londhe SS, Quazi A, Bhosale MG. Exploring the Therapeutic Potential of *Ficus benghalensis* Bark: A Comparative Study of Aqueous and Ethanolic Extracts in Wound Healing. Pharmacog Res. 2027;19(2):673-81.