RESEARCH PAPER

Extraction, Isolation, Characterization, and Pharmacological Evaluation of Bioactive Compounds from Ficus benghalensis for Their Wound Healing Potential

Rubeena Parveen[1], Dr. Rizwan Ahmad[2*], Saurabh Sharma[2], Darakhshan G. Bari[2], Sandeep Kumar[2]

[1] Vivek College of Technical Education, Bijnor

[2] School of Pharmacy, Vivek University, Bijnor

[*] Corresponding Author: Dr. Rizwan Ahmad, Professor, School of Pharmacy, Vivek University, Bijnor.

Email: drrizwanvcte@gmail.com

Received: 12th Mar, 2026 | **Revised:** 24th Mar, 2026 | **Accepted:** 14th Apr, 2026 | **Available Online:** 30th Apr, 2026

ABSTRACT

Wound healing is a complex biological process influenced by oxidative stress, inflammation, and microbial infection, often leading to delayed tissue repair. Limitations associated with conventional therapies, including antimicrobial resistance and high cost, necessitate the exploration of plant-based alternatives with multifunctional therapeutic properties. The present study aimed to evaluate the wound healing potential of hydroalcoholic extract of Ficus benghalensis bark (HAFBB) through physicochemical, phytochemical, and in vivo assessments. The extract was prepared using a hydroalcoholic solvent and characterized for physicochemical parameters, phytoconstituents, and TLC profiling. Acute dermal toxicity of the formulated topical gel was evaluated according to OECD guideline 402, confirming its safety. Wound healing activity was assessed using the excision wound model in Wistar rats, along with histopathological examination and estimation of biochemical markers including hydroxyproline, hexosamine, total protein, superoxide dismutase (SOD), catalase, and reduced glutathione (GSH). The extract demonstrated the presence of bioactive compounds such as flavonoids, phenolics, and tannins, which contribute to antioxidant and anti-inflammatory activity. Treatment with HAFBB significantly enhanced wound contraction, collagen synthesis, antioxidant status, and tissue regeneration. Overall, the findings suggest that Ficus benghalensis possesses promising wound healing potential and may serve as a safe and effective herbal therapeutic for tissue repair.

Keywords: Ficus benghalensis, extraction, isolation, wound healing potential.

How to cite this article: Parveen R, Ahmad R, Sharma S, Bari DG, Kumar S. Extraction, Isolation, Characterization, and Pharmacological Evaluation of Bioactive Compounds from Ficus benghalensis for Their Wound Healing Potential. Int J Drug Deliv Technol. 2026;16(41s): 273-283. DOI: 10.25258/ijddt.16.41s.29

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Disruptions to the skin's anatomical and functional integrity brought on by physical, chemical, thermal, or biological assaults are referred to as wounds. Since the skin serves as the body's main defense against environmental damage, dehydration, and microbial invasion, damage to it starts a complicated and well-coordinated healing process that aims to restore tissue structure and function [1]. Trauma, burns, surgery, infections, and systemic illnesses like diabetes mellitus can all result in wounds, which hinder recovery through altered circulation and inflammatory reactions [2,3].

Wounds can be broadly categorized as either acute or chronic based on their healing characteristics. While chronic wounds stay in a protracted inflammatory state, which causes delayed repair and increased susceptibility to infection, acute wounds heal according to a predictable pattern [3,5]. Hemostasis, inflammation, proliferation, and remodeling are the four overlapping stages of the highly controlled biological process that is wound healing. Clot formation and growth factor release are part of hemostasis, which is followed by inflammation marked by immune cell infiltration and microbial clearance. Fibroblast proliferation, collagen production, angiogenesis, and re-epithelialization are all part of the proliferative phase, whereas remodeling increases tissue strength by rearranging collagen [4,6].

Numerous systemic and local factors affect how well wounds heal. Tissue healing is severely hampered by infection, oxidative stress, malnutrition, age, and systemic diseases [2]. While modest quantities of reactive oxygen species (ROS) aid in cellular signaling and antimicrobial defense, excessive ROS oxidatively damages cellular components, delaying wound healing [11]. Thus, enzymatic and non-enzymatic antioxidant defense systems are crucial for preserving redox equilibrium and promoting tissue regeneration. Antibiotics, antiseptics, anti-inflammatory drugs, and sophisticated wound dressings are all used in conventional wound care. Despite their widespread usage, these methods have a number of drawbacks, including cytotoxicity, antimicrobial resistance, high treatment costs, and decreased efficacy in chronic wounds [14,15]. Due to these restrictions, alternative treatment approaches that are safer, more affordable, and able to improve several facets of the healing process must be developed.

Because of their many pharmacological characteristics, medicinal plants have become attractive sources of wound-healing medicines. Together, the antioxidant, anti-inflammatory, antibacterial, and collagen-promoting properties of phytochemicals such flavonoids, phenolics, tannins, and terpenoids aid in tissue regeneration and repair [16,18]. These substances improve fibroblast activity and collagen deposition, limit microbial infection, regulate inflammatory responses, and lessen oxidative stress.

Because of their biological activity and structural diversity, natural products continue to be important in the drug discovery process. The significance of plants as possible therapeutic agents is shown by the fact that many clinically utilized medications are derived from them [22, 23]. Furthermore, ethnomedical knowledge guides scientific research for the creation of innovative wound healing formulations by offering insightful information on historically utilized botanicals [17].

A medicinal shrub called Mimosa tenuiflora has long been used to treat burns, wounds, and skin diseases. It has bioactive components that are known to have anti-inflammatory, antibacterial, and antioxidant qualities, including as flavonoids, tannins, and phenolic compounds. Its possible function in encouraging wound healing is suggested by these pharmacological activities [21].

Ficus benghalensis

Native to the Indian subcontinent, Ficus benghalensis L. (Moraceae) is a huge evergreen tree with a wide canopy spread and aerial prop roots that generate secondary trunks. The plant yields fruits that resemble syconium and releases a milky latex that has historically been used to treat wounds [24].

Vernacular Names and Taxonomy

Kingdom Plantae; Genus Ficus; Family Moraceae; Species F. benghalensis. Banyan (English), Bargad (Hindi), Vad (Marathi), and Nyagrodha (Sanskrit) are common names [25].

Microscopy and Morphology

A large tree with globose figs, leathery ovate leaves, and greyish-brown bark. Latex and aerial roots are distinctive characteristics. Pharmacognostic identification is supported by microscopy, which reveals paracytic stomata, calcium oxalate crystals, vascular tissues, and laticiferous elements [26].

Parts Used and Conventional Significance

Because of their astringent and protecting properties, bark and latex are mostly utilized for wound healing, whilst leaves and roots are employed for metabolic and inflammatory diseases [27].

Distribution

often found in tropical areas, particularly in India, and able to adapt to a variety of climatic circumstances [28]

The Basis of Wound Healing in Phytochemistry

The plant has phenolic chemicals, flavonoids, tannins, and saponins: • Phenolics and flavonoids: antioxidants (oxidative stress) • Tannins are astringent, which causes wound constriction.[29].

3. MATERIALS ANS METHODS

MATERIALS

A certified botanist verified the authenticity of fresh Ficus benghalensis bark that was gathered from Pune, Maharashtra, India. Phytochemical analysis and extraction were carried out using ethanol and other analytical-grade chemicals. For the gel formulation, triethanolamine, propylene glycol, and carbopol 940 were utilized. Thin layer chromatography was performed using silica gel 60 $F_2{}^1$ plates. Every reagent utilized for phytochemical screening, biochemical estimates, and physicochemical assessment was of standard laboratory grade.

Plant Materials and Verification

Ficus benghalensis bark was gathered from Pune, Maharashtra, India, and verified by a licensed botanist. A sample voucher was placed for future use.

Extraction

Bark that had been shade-dried for 10–12 days at 25–30°C was ground into a powder and Soxhlet extracted using a hydroalcoholic solvent (ethanol:water, 70:30 v/v). The semi-solid extract (HAFBB), which was kept at 2–8°C, was obtained by concentration under lower pressure after extraction was carried out until exhaustion [30].

Physicochemical Assessment

To guarantee the identification, purity, and quality of the crude medication, standard pharmacognostic measures such as ash values (total, acid-insoluble, and water-soluble), extractive values, and loss on drying were established [31].

Ash Values

To evaluate inorganic content and contamination levels, established techniques were used to estimate total ash, acid-insoluble ash, and water-soluble ash [31]

Drying Loss

The sample was dried at 105°C to a constant weight in order to calculate the moisture content, which was then represented as a percentage of weight per weight [32].

Values That Are Extractive

The presence of polar and semi-polar phytoconstituents was estimated using extractive values that were soluble in water and alcohol [33].

Fluorescence Analysis

In order to assess distinctive fluorescence behavior for identification, powdered medication was treated with several reagents and studied under UV light (254 and 366 nm) [34].

Qualitative Screening for Phytochemicals

Alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, and terpenoids were among the principal phytoconstituents that were first identified using conventional assays based on color change or precipitate formation [35].

Thin-layer chromatography (TLC)

TLC was carried out on silica gel 60 $F_{2}{}^{2}{}_{4}$ plates with a mobile phase of ethyl acetate:methanol:water (100:13.5:10). Following derivatization, spots were visible under UV light, and Rf values were noted for phytochemical profiling [36].

Columns Chromatography

Gradient elution and silica gel column chromatography were used for isolation. TLC was used to monitor the fractions, and related fractions were combined and concentrated for additional analysis [37].

FTIR Analysis

To detect functional groups like hydroxyl, carbonyl, and aromatic compounds contained in the isolated fraction, FTIR spectroscopy (4000–400 cm^{-1}) was carried out using the KBr pellet method [38].

***In-vivo* Studies**

Animal Ethics Committee (IAEC) Approval

All experimental procedures involving animals were conducted in accordance with the ethical guidelines for the care and use of laboratory animals as prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Invitox R and D Institute, Pune, under approval number IRDI/2025-26/M6/06. All efforts were made to minimize animal suffering and to reduce the number of animals used in the study.

Experimental Animals

A certified and registered laboratory animal supplier provided healthy adult Wistar albino rats of any sex, weighing between 180 and 220 g. The animals were kept in the institutional animal facility under conventional environmental conditions, which included a 12-hour light-dark cycle, a controlled temperature of 22 ± 2°C, and a relative humidity of 50–60%. To maintain hygienic conditions, rats were housed in sterile polypropylene cages with sterile rice husk bedding that was changed on a regular basis. For the duration of the study, the animals were given a regular pellet meal and unlimited access to water. To reduce stress and guarantee physiological stability, all animals were acclimated to laboratory conditions for at least seven days prior to the start of the experiment. Throughout the course of the trial, animals were handled with care and kept in uniform conditions

Formulation of Gel

Ficus benghalensis bark hydroalcoholic extract (HAFBB) was combined with propylene glycol, triethanolamine, and Carbopol 940 (1% w/w) to create a homogenous topical gel (0.5% w/w extract).

Acute Toxicity to the Skin

Dermal toxicity was assessed at 2000 mg/kg in accordance with OECD 402. The formulation's safety was confirmed by monitoring the animals for irritation, behavioral changes, and mortality over a 14-day period [39].

Excision Wounds Model

Under anesthesia, full-thickness excision incisions (~300–350 mm²) were made. Topical treatments were administered once a day. On days 0, 3, 6, 9, 12, and 15, the wound area was measured; the percentage of wound contraction and the epithelialization period were computed [40].

Experimental Design

Group	Treatment	Dose / Treatment Details
Group I	Normal Control	Saline (10 ml/kg, p.o.)
Group II	Gel Base Control	Gel base (topical, ~0.5 g/wound/day)
Group III	Standard Treatment (Megaheal Gel)	Megaheal gel (topical application)
Group IV	HAFBB Gel (5% w/w)	5% w/w gel (topical application)
Group V	HAFBB Gel (10% w/w)	10% w/w gel (topical application)

Biochemical Evaluation

Granulation tissue was examined for:

• Hydroxyproline (content of collagen)

• Hexosamine (components of the matrix) The total amount of protein • Antioxidant indicators SOD, Catalase, and GSH

These metrics show the state of oxidative stress, tissue regeneration, and collagen formation [41].

Histopathology

Collagen deposition, fibroblast proliferation, angiogenesis, inflammation, and re-epithelialization were assessed in tissue sections (stained with H&E) [42].

Statistical Analysis

Dunnett's test and one-way ANOVA were used to examine the data, which were represented as mean ± SEM. The threshold for significance was fixed at $p < 0.05$ [43].

RESULTS

Plant collection and authentification

Ficus benghalensis bark was gathered and taxonomically verified at Pune, Maharashtra, India. A sample voucher was kept for further use.

Extraction Yield (%)

A dark brown semi-solid extract (HAFBB) was produced by hydroalcoholic extraction of the dried bark using a Soxhlet apparatus (ethanol:water, 70:30 v/v). The excellent extraction of polar and semi-polar elements was demonstrated by the yield percentage of 8.75%.

Table 1: Percentage Yield of Extract

Extract	Solvent	Yield (%)	Appearance
HAFBB	**Ethanol:Water (70:30)**	**8.75**	**Dark brown semi-solid**

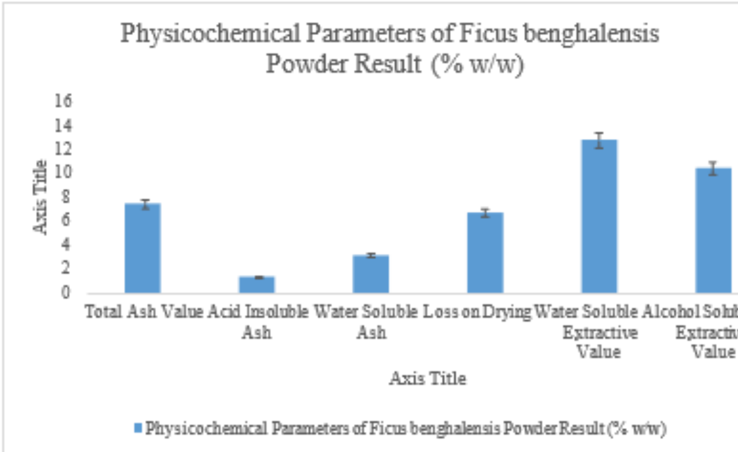
Physicochemical Assessment

The purity and quality of the crude medication were evaluated using physicochemical criteria such as ash values, loss on drying, and extractive values. The results show acceptable moisture and inorganic content limitations, indicating that the plant material is suitable for additional pharmacological testing.

Table 2: Physicochemical Parameters of *Ficus benghalensis* Powder

Sr. No.	Parameter	Result (% w/w)
1	Total Ash Value	7.48 ± 0.12
2	Acid Insoluble Ash	1.36 ± 0.05
3	Water Soluble Ash	3.14 ± 0.09
4	Loss on Drying	6.72 ± 0.11
5	Water Soluble Extractive Value	12.83 ± 0.15
6	Alcohol Soluble Extractive Value	10.47 ± 0.13

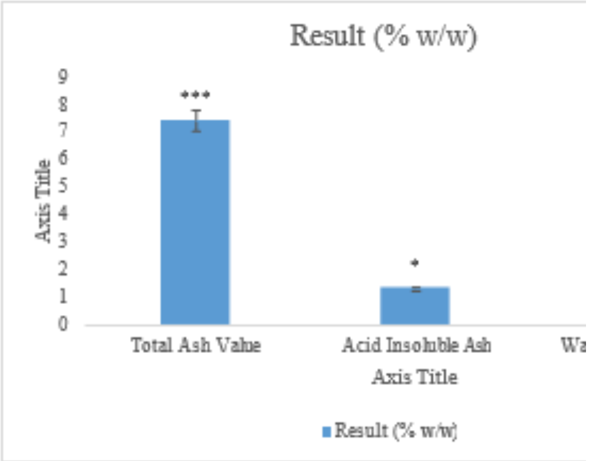
Values are expressed as Mean ± SEM (n = 3)



Graph 1: Physicochemical Parameters of *Ficus benghalensis* Powder

Ash Values

To evaluate the cleanliness and inorganic content of Ficus benghalensis bark, ash values were calculated. While the low acid-insoluble ash (1.36 ± 0.05% w/w) indicates minor siliceous contamination, such as sand and soil, the total ash value (7.48 ± 0.12% w/w) reflects overall mineral composition. The existence of soluble inorganic components is indicated by the water-soluble ash (3.14 ± 0.09% w/w). Overall, the findings show that the crude medicine is suitable for additional investigation and has an acceptable level of purity.



Graph 2: Ash Values of *Ficus benghalensis* Bark Powder

Loss of Drying

The moisture content of Ficus benghalensis bark was estimated by calculating the loss upon drying. The obtained value of 6.72 ± 0.11% w/w suggests controlled moisture levels, indicating good stability of the crude medication with low danger of microbial development or active ingredient degradation.

Extractive Values

To determine whether soluble phytoconstituents were present, extractive values were assessed. The water-soluble extractive value (12.83 ± 0.15% w/w) was higher than the alcohol-soluble extractive value (10.47 ± 0.13% w/w), suggesting that hydroalcoholic extraction is appropriate since it contains a higher proportion of polar elements like glycosides and phenolics.

Fluorescence Analysis

When Ficus benghalensis powder was treated with various chemicals, fluorescence examination revealed distinctive color changes under visible and UV light (254 and 366 nm). These unique fluorescence patterns enhance the pharmacognostic standardization of the crude medication by confirming its authenticity and indicating the presence of various phytoconstituents.

Phytochemicals Screening

Alkaloids, glycosides, flavonoids, tannins, phenolic compounds, saponins, and carbohydrates were found in the hydroalcoholic extract (HAFBB) of Ficus benghalensis, according to preliminary phytochemical study; steroids, terpenoids, and proteins were not. Strong antioxidant capacity is suggested by the predominance of flavonoids and phenolic substances, while astringent and collagen-promoting qualities are indicated by tannins and saponins, which together boost the extract's ability to heal wounds.

Thin Layer Chromatography (TLC) Analysis

Ethyl acetate:methanol:water (100:13.5:10) TLC examination of HAFBB revealed a clear spot with an Rf value of 0.72, suggesting the presence of a significant phytoconstituent. The observed separation validates the presence of active chemicals, most likely polyphenolic in nature, that support the extract's wound-healing and antioxidant qualities.

Studis in vivo

Acute Dermal Toxicity

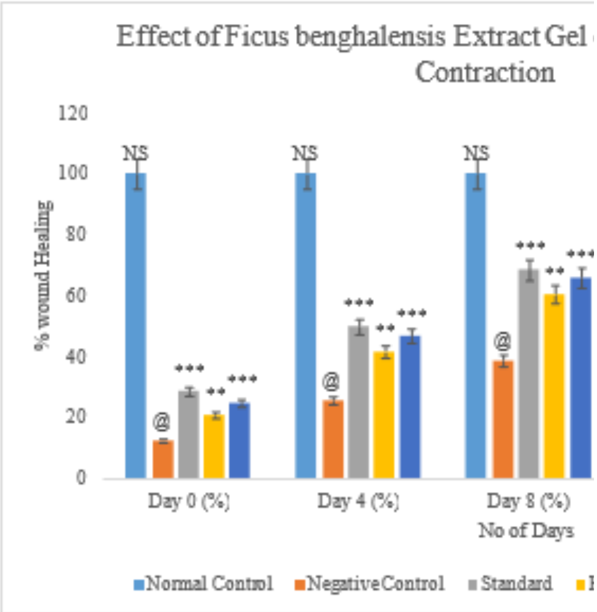
At doses of 100–2000 mg/kg over 14 days, acute cutaneous toxicity of the HAFBB gel (OECD 402) did not exhibit any symptoms of erythema, edema, irritation, behavioral abnormalities, or death. All groups showed good dermal tolerance as the application site remained normal. These results support the formulation's viability for further wound healing research by establishing it as safe for topical usage up to 2000 mg/kg.

Excision Wound Healing

Using the excision wound model in Wistar rats, the gel formulation containing the hydroalcoholic extract (HAFBB) of Ficus benghalensis was assessed for its ability to cure wounds. By calculating the percentage of wound contraction at various time intervals (days 0, 4, 8, 12, and 15), the wound healing process was evaluated.

Table 3: Effect of *Ficus benghalensis* Extract Gel on Percentage Wound Contraction

Group	Treatment	Day 0 (%)	Day (%)
Normal Control	Saline	NA	NA
Negative Control	Gel base	12.4 ± 1.2	25.6 ± 1.8
Standard	Megaheal gel	28.6 ± 1.5	49.8 ± 1.9
HAFBB (5%)	5% w/w gel	20.8 ± 1.4	41.6 ± 1.8
HAFBB (10%)	10% w/w gel	24.7 ± 1.5	46.8 ± 2.0



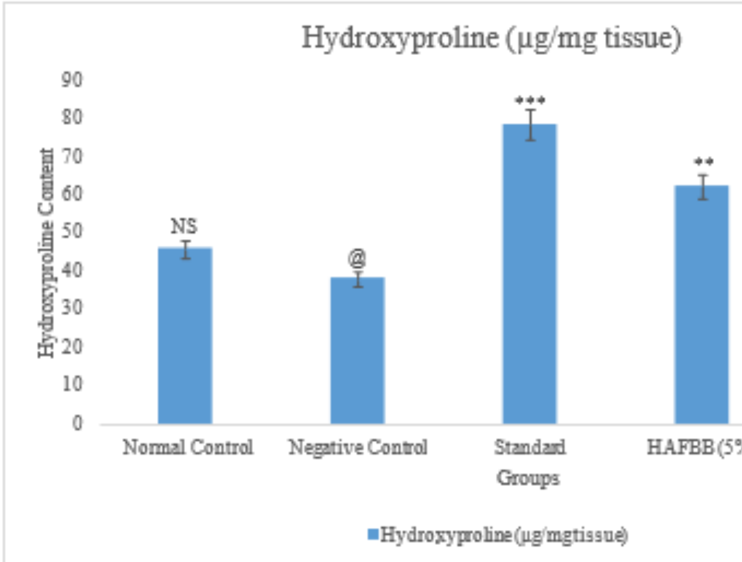
Graph 3: Effect of *Ficus benghalensis* Extract Gel on Percentage Wound Contraction

Biochemical Estimation

After HAFBB treatment, collagen synthesis and antioxidant status in wound tissue were assessed using biochemical analysis.

Hydroxyproline Content

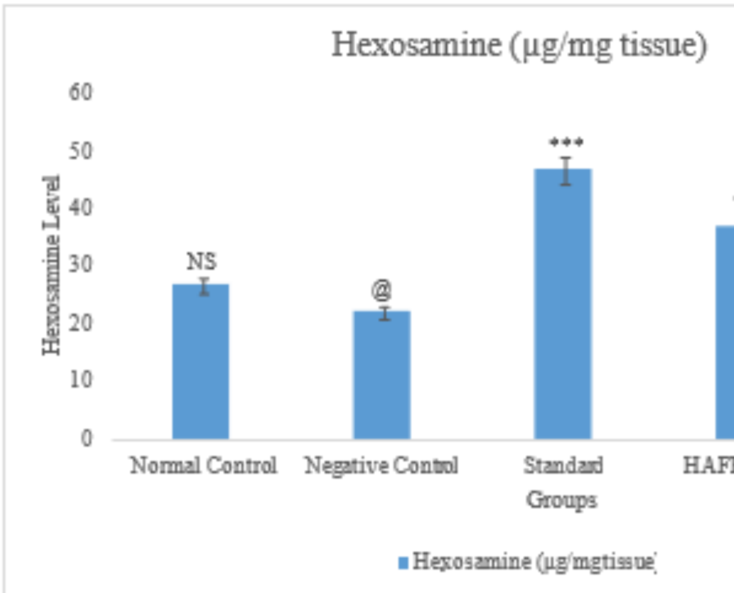
When compared to the negative control, the extract-treated groups' hydroxyproline levels were noticeably higher, suggesting improved collagen production. Collagen content was higher in the 10% HAFBB group (72.16 ± 2.08 µg/mg) than in the 5% group (62.48 ± 1.95 µg/mg), and it was closer to the standard (78.63 ± 2.14 µg/mg) than in the negative control group (38.42 ± 1.82 µg/mg). These results validate that HAFBB therapy improves extracellular matrix production and speeds up wound healing.



Graph 4: Effect of *Ficus benghalensis* Extract on Hydroxyproline Content

Hexosamine Content

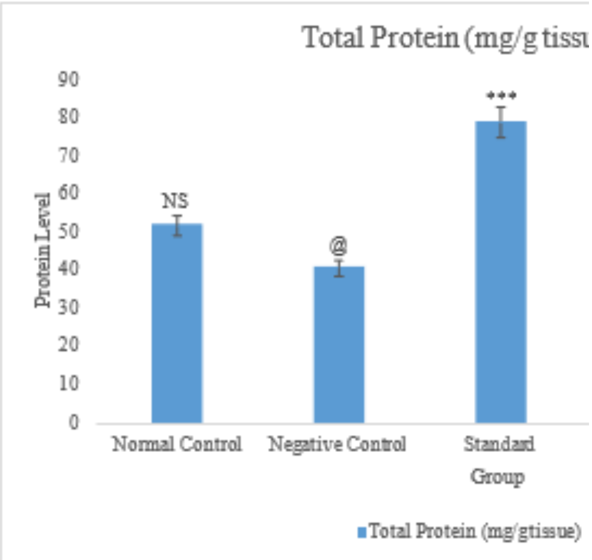
Hexosamine levels were higher in HAFBB-treated groups than in the negative control, suggesting improved extracellular matrix formation and glycosaminoglycan synthesis. The 10% HAFBB group (42.76 ± 1.46 µg/mg) showed higher levels than the 5% group (36.92 ± 1.28 µg/mg) and was comparable to the standard (46.85 ± 1.69 µg/mg), while the negative control remained lower (22.18 ± 1.07 µg/mg). This implies better wound healing and matrix stabilization.



Graph 5: Effect of *Ficus benghalensis* Extract on Hexosamine Content

Total Protein

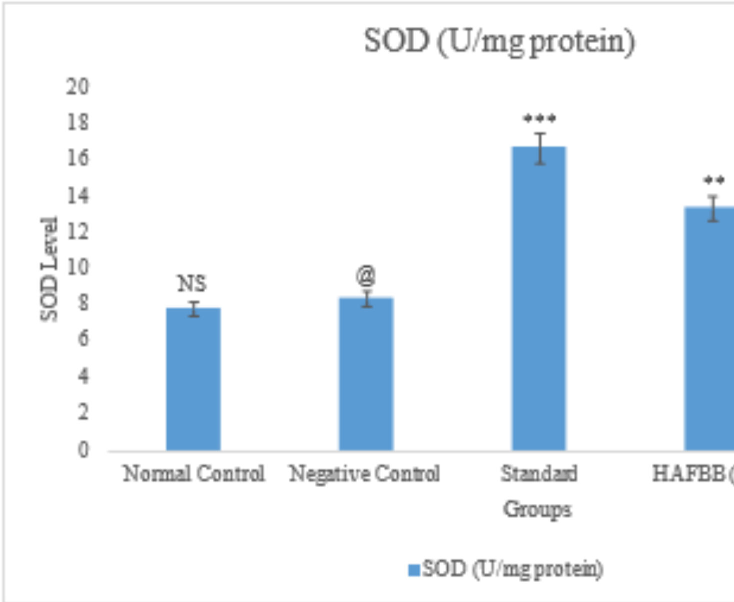
The extract-treated groups' total protein levels were noticeably higher, indicating improved cellular proliferation and tissue regeneration. While the negative control had lower levels (41.28 ± 1.94 mg/g), the 10% HAFBB group (72.94 ± 2.14 mg/g) had higher protein content than the 5% group (63.28 ± 2.08 mg/g) and was closer to the standard (79.56 ± 2.23 mg/g). These findings suggest that HAFBB therapy improves the healing response.



Graph 6: Effect of *Ficus benghalensis* Extract on Total Protein

Superoxide Dismutase (SOD)

HAFBB-treated groups had higher SOD activity (8.42 ± 0.41 U/mg) than the negative control, suggesting better antioxidant defense. Effective scavenging of reactive oxygen species and improved wound healing were suggested by the 10% HAFBB group's increased activity (15.28 ± 0.52 U/mg) compared to the 5% group (13.42 ± 0.48 U/mg) and comparable to the standard (16.75 ± 0.58 U/mg).



Graph 7: Effect of *Ficus benghalensis* Extract on SOD Activity

Catalases Activity

Catalase activity was significantly lower in the negative control (21.45 ± 1.12 U/mg protein) and increased in the groups treated with HAFBB, suggesting better hydrogen peroxide detoxification and less oxidative stress. Effective antioxidant defense and improved healing were demonstrated by the 10% HAFBB group (41.92 ± 1.47 U/mg), which had higher ctivity
than the 5% group (36.28 ± 1.34 U/mg) and was comparable to the standard (45.63 ± 1.58 U/mg)
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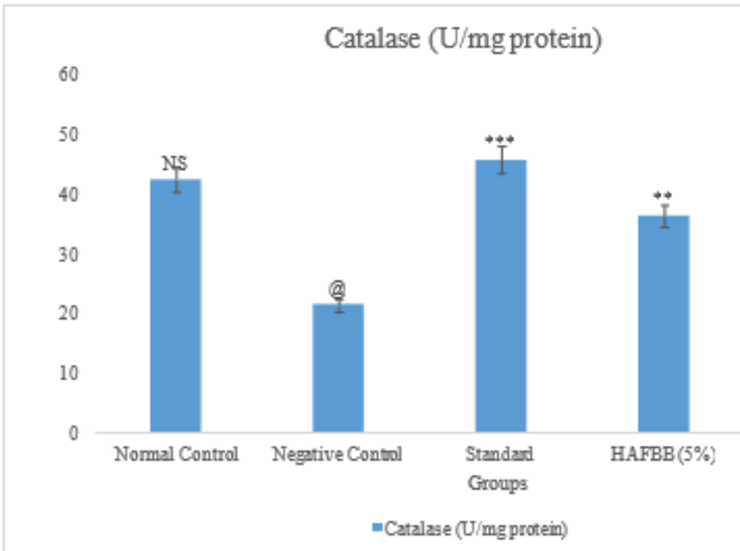
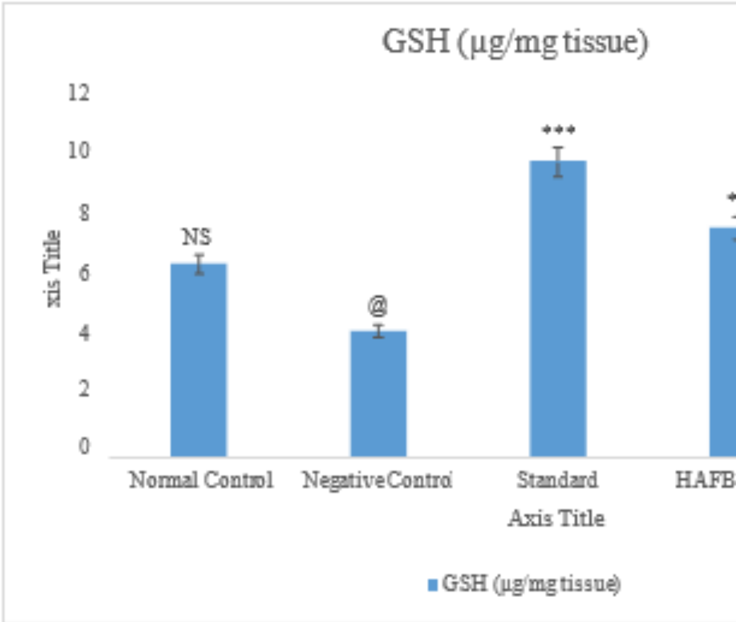


Graph 8: Effect of *Ficus benghalensis* Extract on Catalase Activity

Effect of Treatment on Glutathione (GSH) Level

GSH levels were considerably higher after HAFBB treatment, indicating the recovery of non-enzymatic antioxidant capacity, while they were lower in the negative control (4.18 ± 0.92 µg/mg). Reduced oxidative damage and enhanced tissue repair were supported by the 10% HAFBB group (8.78 ± 1.22 µg/mg), which improved more than the 5% group (7.62 ± 1.10 µg/mg) and nearly reached the standard (9.84 ± 1.34 µg/mg).

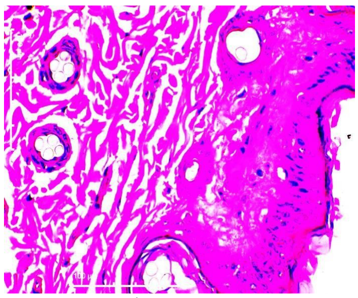


Graph 9: Effect of *Ficus benghalensis* Extract on GSH Level

Histopathological Analysis

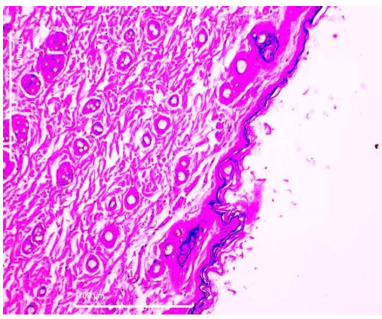
Normal S1 (10X)

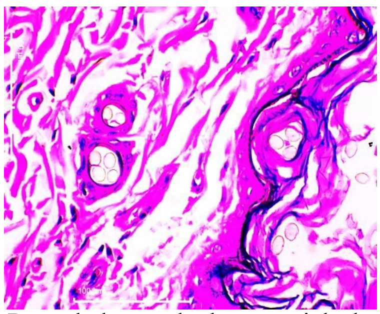
Exhibited a normal structure and architecture. No pathological changes were observed. No inflammatory infiltration. No abnormality detected.



Control S1 (10X)

Control S1 (40X)

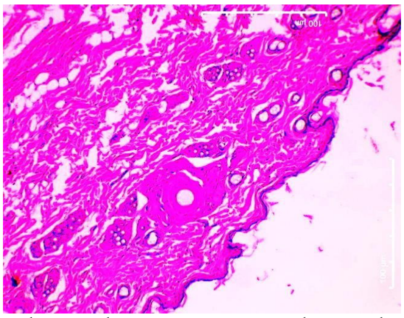




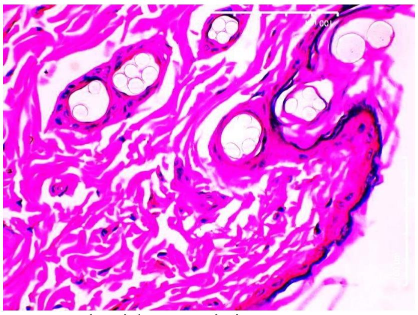
Revealed marked synovial hyperplasia and inflammatory cell infiltration. Structural changes were observed. Severe abnormality were detected.

STD S1 (10X)

Exhibited a normal structure and architecture.

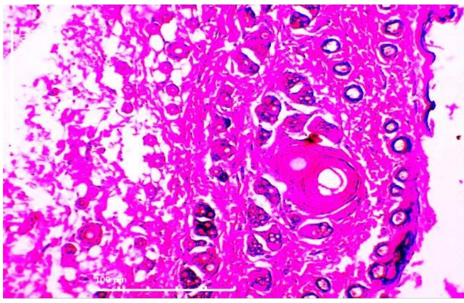


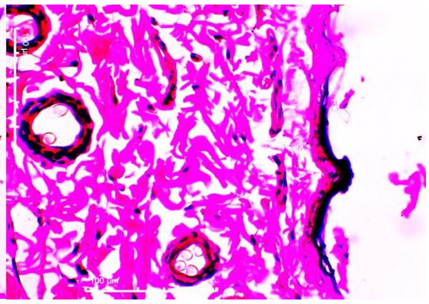
STD S1 (40X)



Mild hemorrhage were observed. No pathological changes were observed. No inflammatory infiltration. No abnormality detected.

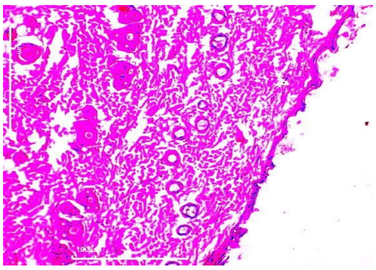
HAFBB (5%) S1 (10X) **HAFBB (5%) S1 (40X)**

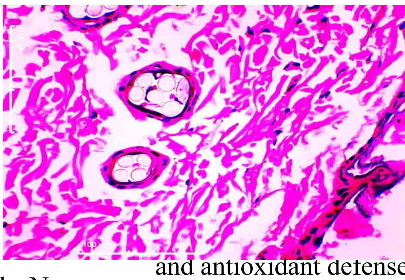




No pathological changes were observed. No inflammatory infiltration. Exhibited a normal structure and architecture. No abnormality detected.

HAFBB (10%) S1 (10X) **HAFBB (10%) (10X)**





Mild structural changes were observed No inflammatory infiltration. Mild abnormality selected.

DISCUSSION

The current study supported the traditional use of the hydroalcoholic extract of Ficus benghalensis bark (HAFBB) by demonstrating its great potential for wound healing. Higher water-soluble extractives demonstrated the predominance of polar bioactive components, while physicochemical metrics validated the crude drug's purity and quality. Flavonoids, phenolics, tannins, and saponins were identified by phytochemical analysis. These compounds are known to support collagen production, wound contraction, and antioxidant activity.

It was discovered that the formulation had no skin toxicity and was safe up to 2000 mg/kg. HAFBB considerably increased wound contraction in the excision model in a dose-dependent manner, with activity similar to that of the conventional medication in the 10% formulation.

Increased quantities of protein, hexosamine, and hydroxyproline were found in the biochemical data, suggesting improved tissue regeneration and collagen synthesis. Reduced oxidative stress is also suggested by elevated antioxidant markers (SOD, ...nproved tissue organization, ...ation, and increased ...re all validated by ...s.

..., HAFBB's wound-healing ...ed to its phytochemical ...gether support tissue repair and antioxidant defense.

CONCLUSION

The current work shows that in the excision wound model, the hydroalcoholic extract of Ficus benghalensis bark (HAFBB) significantly promotes wound healing. At greater concentrations, the extract's effects on wound contraction, collagen formation, and antioxidant protection were similar to those of the usual treatment. Topical application of the formulation was determined to be safe.

The inclusion of flavonoids, phenolics, tannins, and saponins is thought to be responsible for the reported action. These compounds work together to support tissue regeneration, extracellular matrix synthesis, and antioxidant activity. These results demonstrate the potential of Ficus benghalensis as a natural and efficient wound healing agent and support its traditional use.

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