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FORMULATION AND EVALUATION OF A POLYHERBAL HYDROGEL OF *FICUS RELIGIOSA* AND *BRYOPHYLLUM PINNATUM* FOR ANTIMICROBIAL AND WOUND-CARE APPLICATIONS

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ABSTRACT

Wound healing is a complex biological process involving inflammation, tissue proliferation, collagen synthesis, angiogenesis, and re-epithelialization. Despite the availability of various wound care therapies, achieving rapid and effective healing with minimal scarring remains a challenge, particularly in chronic and infected wounds. The present study focuses on the formulation of an herbal hydrogel containing extracts of *Ficus religiosa* and *Bryophyllum pinnatum* for potential wound healing applications. These medicinal plants are well known for their antimicrobial, antioxidant, and anti-inflammatory properties due to the presence of bioactive constituents such as flavonoids, tannins, and phenolic compounds. Such phytochemicals may help reduce oxidative stress, inhibit microbial growth, and support tissue regeneration. A hydrogel-based delivery system was selected because of its ability to maintain a moist wound environment, improve drug retention, and enhance patient compliance. Suitable polymers and excipients were incorporated to obtain an effective formulation with desirable physicochemical characteristics. The prepared hydrogel was evaluated for parameters including pH, spreadability, extrudability, and stability to ensure safety and ease of application. The formulation is also expected to provide controlled release of active constituents, thereby prolonging therapeutic action. Although experimental biological studies were not performed, the formulation approach is supported by

previously reported pharmacological activities of the selected plant extracts.

KEYWORDS: Polyherbal hydrogel, *Ficus religiosa, Bryophyllum pinnatum,* wound healing, anti-microbial, anti-oxidant, Franz diffusion, hydrogel formulation.

1. INTRODUCTION

The biological process of the recovery of wounds is complex and involves tissue remodeling, proliferation, and inflammation. Delayed healing in chronic conditions such as diabetes and infections may lead to severe complications. Because hydrogels provide targeted therapeutic benefits, enhance medication penetration, and provide a moist environment, they have become more important in wound care. Hydrogels are regarded as efficient topical drug delivery methods because of their high water-holding capacity, flexibility, biocompatibility, and controlled drug release characteristics.

Herbal extracts of *Ficus religiosa* and *Bryophyllum pinnatum* possess remarkable antimicrobial, antioxidant, anti-inflammatory, and wound-healing activities. These medicinal plants contain important phytoconstituents such as phenolic compounds and flavonoids, which play a major role in tissue repair and skin regeneration. Phenolic compounds help reduce oxidative stress by scavenging free radicals, while flavonoids exhibit antimicrobial and anti-inflammatory properties that help prevent infection and promote faster healing. These compounds also enhance collagen synthesis, epithelialization, and angiogenesis, which are essential for effective wound healing.

The present study focuses on the formulation of a herbal hydrogel incorporating extracts of *Ficus religiosa* and *Bryophyllum pinnatum* to evaluate their combined wound-healing potential. The developed hydrogel is expected to provide enhanced therapeutic efficacy, reduced microbial load, improved tissue regeneration, and a safe natural alternative for the treatment of wounds, burns, and skin infections.[1]

1.1 Need of herbal systems

Herbal wound healing systems are gaining importance due to their natural origin, therapeutic potential, and reduced side effects compared to some conventional treatments. The need for herbal systems in wound healing includes.

a) Promotion of Faster Wound Healing: Many medicinal plants contain bioactive compounds such as flavonoids, tannins, alkaloids, and phenolic compounds that enhance

tissue regeneration and accelerate wound closure.

b) Antimicrobial Activity: Herbal extracts possess antibacterial, antifungal, and antiviral properties that help prevent wound infections and support a healthy healing environment.

c) Anti-inflammatory Effects: Natural phytoconstituents reduce inflammation at the wound site, minimizing pain, swelling, and tissue damage during the healing process.

d) Biocompatibility and Safety: Herbal systems are generally well tolerated, biodegradable, and less likely to cause adverse reactions when appropriately formulated.

e) Cost-Effectiveness: Medicinal plants are often readily available and economical, making herbal wound care products affordable and accessible, especially in resource-limited settings.

f) Growing Demand for Natural Therapies: Increasing awareness and preference for natural and plant-based healthcare products have encouraged the development of herbal wound healing formulations.[2]

1.2 Overview of plants

Fig 1: Leaves of *Ficus religiosa*.

Ficus religiosa (the sacred fig or Peepal tree) supports wound healing through bioactive secondary metabolites like flavonoids, phenolics, and saponins. These phytochemicals accelerate tissue repair, stimulate collagen synthesis, and provide strong antimicrobial defense against pathogens like *Staphylococcus aureus*, *E. coli*, and *Candida albicans*.[3]

Fig 2: Leaves of *Bryophyllum pinnatum*.

Bryophyllum pinnatum often referred to as the miracle leaf or Patharchatta, is a powerful medicinal plant. Its leaf extracts are frequently utilized to speed up skin healing in both conventional and contemporary herbal treatments. Its antibacterial and wound-healing qualities are mostly attributed to flavonoids and phenolics. They achieve this through potent antioxidant, anti-inflammatory, and tissue-regenerating mechanisms that combat infections and accelerate cellular repair.[4]

1.3 Rationale for synergistic combination

Wound healing is a complex biological process often affected by delayed tissue regeneration, persistent inflammation, and microbial infection. *Ficus religiosa* possesses significant antimicrobial, antioxidant, and anti-inflammatory properties that help prevent wound infections and promote healing. Its bioactive constituents stimulate fibroblast proliferation, collagen synthesis, and angiogenesis, which are essential for tissue regeneration. However, higher concentrations may sometimes affect normal tissue repair.

Bryophyllum pinnatum is well known for its regenerative, anti-inflammatory, and antioxidant activities. When combined with *Ficus religiosa*, it enhances tissue repair while supporting safer and more effective antimicrobial action at lower concentrations. This synergistic combination helps control infection, reduce oxidative stress, and accelerate wound healing.

Incorporation of both extracts into a hydrogel system further improves their therapeutic potential by enhancing skin penetration, maintaining a moist wound environment, and providing sustained release of active constituents. Hydrogels also improve patient compliance due to their non-greasy nature and ease of application.

Thus, the combination of *Ficus religiosa* and *Bryophyllum pinnatum* in a hydrogel formulation represents a promising herbal approach for effective wound management and tissue regeneration.

1.4 Overview of Hydrogel

Hydrogels have become a key component in the development of biomaterial science, transforming applications in a wide range of biological domains because of their distinctive three-dimensional networks of hydrophilic polymers. These networks stand out for their amazing capacity to enlarge without dissolving, preserving structural integrity through chemical or physical cross-linking processes. Hydrogels are especially well-suited for uses in

drug delivery systems, tissue engineering, wound healing, and other fields because of their inherent ability to replicate the physicochemical characteristics of the natural extracellular matrix. The origins of hydrogel research and its expansion into the biomedical sciences are excellent illustrations of an inventive path, showcasing the materials' versatility in addressing difficult biological issues and their contribution to the development of biomedical solutions.[5]

1.5 Advantages of Hydrogel

a) Maintains a Moist Wound Environment: Hydrogels retain a high amount of water, preventing wound dehydration and creating optimal conditions for healing.

b) Promotes Faster Healing: The moist environment supports cell migration, tissue regeneration, and re-epithelialization, accelerating the healing process.

c) Provides Cooling and Soothing Effect: Hydrogels offer immediate relief from pain, irritation, and burning sensations due to their high-water content.

d) Biocompatible and Non-Toxic: Most hydrogel-forming polymers are biocompatible, minimizing the risk of tissue irritation and adverse reactions.

e) Provides a Protective Barrier: They protect the wound from external contaminants and microbial invasion, reducing the risk of infection.

f) Easy Application and Removal: Hydrogels are soft, flexible, and can be removed without causing trauma to newly formed tissue.

g) Suitable for Incorporating Herbal Extracts: Hydrogels serve as excellent carriers for plant-derived bioactive compounds, enhancing their stability, retention, and therapeutic efficacy at the wound site.[6]

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

Leaves of *Ficus religiosa* and *Bryophyllum pinnatum* were procured from the local geographical region during the appropriate vegetative stage. The collected plant materials were examined carefully to ensure the absence of microbial contamination, insect infestation, and mechanical deterioration. Subsequently, the samples were thoroughly rinsed with

distilled water to eliminate extraneous matter and adhered impurities. The cleaned leaves were subjected to shade drying under controlled environmental conditions to prevent degradation of thermolabile phytoconstituents. After complete desiccation, the dried materials were pulverized separately using a mechanical grinder to obtain coarse powder and stored in airtight containers for subsequent experimental procedures.

Botanical authentication of the collected plant specimens was performed by a qualified taxonomist on the basis of macroscopic and morphological characteristics. The authentication process ensured the taxonomical identity, purity, and authenticity of the plant materials employed in the present investigation. Voucher specimens were prepared and preserved in the departmental herbarium for future reference and documentation. The authenticated powdered materials were further utilized for extraction and formulation of the herbal hydrogel preparation.

2.2 Phytochemical Screening of *Ficus religiosa* and *Bryophyllum pinnatum* extracts

The phytochemical screening of *Ficus religiosa* and *Bryophyllum pinnatum* leaf extracts was carried out using standard qualitative chemical tests to identify the presence of various bioactive constituents. These phytochemicals, including flavonoids, alkaloids, glycosides, tannins, steroids, and terpenoids, are known to contribute to the therapeutic and wound-healing properties of medicinal plants. Different reagents were employed to detect specific classes of phytoconstituents based on characteristic colour changes or precipitate formation.[6][7]

Table 1: Phytochemical screening tests performed for *Ficus religiosa* and *Bryophyllum pinnatum* leaf extracts.

Sr. no.	Test	Procedure	Observation
1.	NaOH / Alkaline Reagent Test	Extract was treated with NaOH followed by a few drops of HCl.	Appearance of yellow colour which disappeared on addition of HCl indicated flavonoids.
2.	Lead Acetate Test	Extract was treated with lead acetate solution.	Formation of yellow precipitate confirmed flavonoids.
3.	Pew's Test	Extract was treated with zinc powder and concentrated H_2SO_4.	Development of red or orange colour indicated flavonoids.
4.	Dragendorff's Test	Extract was treated with Dragendorff's reagent.	Formation of orange or reddish-brown precipitate indicated alkaloids.
5.	Wagner's Test	Extract was treated with Wagner's reagent.	Formation of brown or reddish precipitate indicated alkaloids.
6.	Tannic Acid	Extract was heated with water and	Formation of precipitate

		treated with lead acetate solution.	confirmed glycosides.
7.	Liebermann–Burchard Test	Extract was mixed with chloroform and acetic acid, heated, and concentrated H_2SO_4 was added.	Development of green or bluish-green colour indicated steroids.
8.	Salkowski Test	Extract was mixed with chloroform and concentrated H_2SO_4 was carefully added.	Formation of a reddish-brown ring at the interface confirmed terpenoids.

2.3 Extraction and concentration of extracts

2.3.1 Soxhlet Extraction of *Ficus religiosa*

The ethanolic extract of *Ficus religiosa* leaves was prepared using the Soxhlet extraction method. Initially, dried leaves of *Ficus religiosa* were finely powdered, and approximately 25 g of the powdered material was accurately weighed using an electronic balance. The weighed powder was then packed into a muslin cloth or extraction thimble and carefully placed inside the Soxhlet extractor. A sufficient volume of ethanol was added to a round-bottom flask, which served as the extraction solvent. The Soxhlet apparatus was assembled properly, and the solvent was heated to its boiling point. As the ethanol vaporized, it condensed in the condenser and continuously percolated through the plant material, facilitating the extraction of bioactive constituents. This process was allowed to continue for approximately 8–12 extraction cycles, corresponding to about 6–8 hours, until the solvent draining through the siphon tube became nearly colorless, indicating exhaustive extraction of the phytoconstituents. Upon completion of the extraction process, the ethanolic extract collected in the round-bottom flask was concentrated by removing the solvent using a rotary evaporator. In the absence of a rotary evaporator, concentration was carried out using a water bath maintained at 50–60°C to prevent thermal degradation of the active compounds. The concentrated crude extract obtained was then collected and further dried, if necessary, to remove any residual solvent. Finally, the dried extract was transferred into an airtight container and stored under appropriate conditions until further phytochemical analysis and formulation studies were carried out.[8]

Fig 3: Soxhlet Extraction of *Ficus religiosa.*

2.3.2 Maceration of *Bryophyllum pinnatum*

Fresh leaves of *Bryophyllum pinnatum* were collected and thoroughly washed with distilled water to remove adhering dust, dirt, and other impurities. The cleaned leaves were then shade-dried for several days to prevent degradation of heat-sensitive phytoconstituents and to reduce moisture content. Once completely dried, the leaves were finely powdered using a mechanical grinder to increase the surface area for efficient extraction. Approximately 10 g of the powdered leaf material was accurately weighed and transferred into a clean 250 mL beaker. To this, 100 mL of distilled water was added as the extraction solvent. The mixture was subjected to heating on a heating mantle equipped with an energy regulator, maintaining a controlled temperature throughout the process. The contents were stirred intermittently to facilitate uniform extraction and enhance the release of bioactive compounds into the solvent through maceration. After sufficient heating, the mixture was allowed to cool to room temperature and subsequently filtered through filter paper to separate the liquid extract from the plant residues. The clear filtrate obtained was collected as the crude aqueous leaf extract of *Bryophyllum pinnatum* and stored in a clean, airtight container for further phytochemical screening and antimicrobial evaluation.[**Error! Bookmark not defined.**]

Fig 4: Maceration of *Bryophyllum pinnatum.*

2.4 Estimation of Total Phenolic Content (TPC)

2.4.1 Preparation of Folin–Ciocalteu Reagent (10% v/v)

Folin–Ciocalteu reagent was prepared by diluting 1 mL reagent with distilled water to 10 mL (1:10 dilution).

2.4.2 Preparation and Analysis of Standard (Gallic Acid)

A stock solution of gallic acid (100 µg/mL) was prepared by dissolving 10 mg of gallic acid in methanol and making up the volume to 100 mL. From this stock solution, working standard solutions of 10, 20, 30, 40, and 50 µg/mL were prepared by suitable dilution.

Procedure: 1 mL of each standard solution, 3 mL of distilled water, and 0.5 mL of (10% v/v) Folin–Ciocalteu reagent were combined for the experiment. Following three to five minutes of standing, 2 mL of (7.5% w/v) sodium carbonate solution was added, and distilled water was used to bring the volume up to 10 mL. For 30 to 60 minutes, the mixes were allowed to develop color at room temperature. A UV-Visible spectrophotometer was then used to detect the absorbance at 765 nm. Plotting absorbance versus concentration allowed for the creation of a standard gallic acid calibration curve.[9]

2.4.3 Preparation of Sample Extracts

Preparation of Stock Solutions: The ethanolic extract of *Ficus religiosa* was prepared by dissolving 10 mg of the extract in 10 mL of methanol to obtain a stock solution of 1000 µg/mL. Similarly, the aqueous extract of *Bryophyllum pinnatum* was prepared by dissolving 10 mg of the extract in 10 mL of methanol to obtain a concentration of 1000 µg/mL. For the combined extract, equal volumes of the *F. religiosa* and *B. pinnatum* stock solutions were mixed in a 1:1 ratio to obtain a final concentration of 1000 µg/mL.

Preparation of Working Solutions: Working solutions of *F. religiosa* were prepared at concentrations of 10, 20, 30, 40, and 50 µg/mL by suitable dilution of the stock solution. For *B. pinnatum* and the combined extract, working solutions of 20, 40, 60, 80, and 100 µg/mL were prepared.

Reaction procedure: For the estimation of total phenolic content, 1 mL of each sample solution was taken in a test tube and mixed with 3 mL of distilled water. To this, 0.5 mL of diluted Folin–Ciocalteu reagent (10% v/v) was added and the mixture was allowed to stand for 5 minutes. Then, 2 mL of sodium carbonate solution (7.5% w/v) was added, and the volume was made up to 10 mL with distilled water. The mixture was incubated at room

temperature for 30–60 minutes for color development. The absorbance of the resulting solution was measured at 765nm spectrophotometrically.[10]

***Fig 5a: Stock solutions of* B. pinnatum.**

Fig 5b: Stock solutions of *F. religiosa.*

Fig 5c: Stock solutions of combined extracts.

2.4.4 Blank

The blank solution was prepared using distilled water in place of the sample extract and was used as a reference for absorbance measurement at 765 nm.

2.4.5 Calibration Curve

A calibration curve was prepared by plotting gallic acid concentration (µg/mL) on the X-axis against the corresponding absorbance values on the Y-axis. The curve was used to calculate the total phenolic content of the extracts.

TPC values below the limit of quantification were considered negligible and reported as zero.

2.4.6 Calculation - Total Phenolic Content (TPC)

$$TPC = \frac{C \times V \times D}{W}$$

Where: C = concentration from calibration curve (µg/mL)

V = volume of extract (mL)

D = dilution factor

W = weight of sample (g)

Expressed as: mg Gallic Acid Equivalent (GAE)/g extract

2.5 TLC analysis

2.5.1 TLC of isolated *Ficus religiosa* extract

Procedure: TLC was performed on pre-coated aluminumplates of silica gel 60 as stationary phase. The samples (plant extract) were applied as small spots using a capillary tube. The plates were developed in a mobile phase consisting of: n-butanol: acetic acid: water (14:3:3). The plates were then sprayed with 5% alcoholic KOH reagent. After development, the plates were air-dried and visualized under UV light.[11]

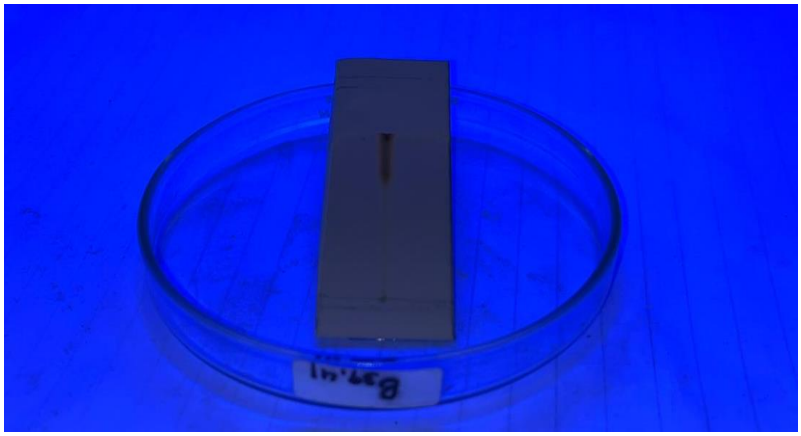

Fig 6: TLC Profile of isolated *F. religiosa* extract under long UV Light (665nm)

2.5.2 TLC of isolated *Bryophyllum pinnatum* extract

Procedure: TLC was performed on pre-coated aluminum plates of silica gel 60 as stationary phase. The samples (plant extract) were applied as small spots using a capillary tube. The plates were developed in a mobile phase consisting of: ethyl acetate: formic acid: methanol: water (10: 0.5: 0.6: 0.2)**.** After development, the plates were sprayed with Natural product reagent A air-dried and visualized under UV light.[12]

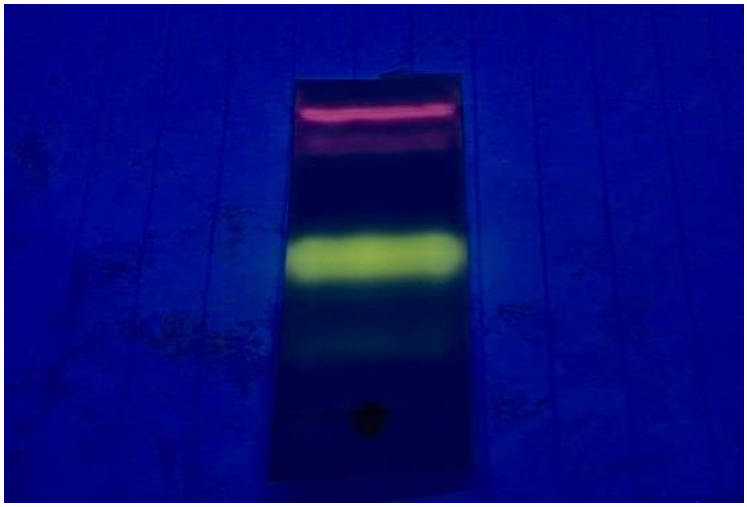

Fig 7: TLC Profile of isolated *B. pinnatum* extract under long UV Light (665nm).

2.5.3 Retardation factor

The retardation factor (Rf) was calculated using the formula:

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

2.6 Identification of Bacterial Strains (Gram Staining)

Pure bacterial isolates were identified using the Gram staining technique.

Procedure: A thin bacterial smear was prepared on a clean glass slide using a sterile loop. The smear was allowed to air dry and then heat-fixed by passing the slide through a flame to

ensure proper adherence of the bacterial cells. The smear was flooded with crystal violet and allowed to stand for 1 minute, followed by rinsing with water. Gram's iodine was then applied for 1 minute to form a crystal violet–iodine complex within the bacterial cells. Decolorization was carried out using ethanol to differentiate Gram-positive and Gram-negative bacteria, and the slide was immediately rinsed with water. Subsequently, the smear was counterstained with safranin for 1 minute, rinsed, and air-dried. The stained slide was finally examined under a microscope at 10× magnification for bacterial identification based on staining characteristics.[13]

Fig 8: Gram staining.

2.7 Determination of Minimum Inhibitory Concentration (MIC) of Combined Plant Extracts

Determined by broth dilution or tube dilution method.

Procedure: Nutrient broth was prepared by dissolving 0.5 g in 25 mL distilled water and dispensed into sterile test tubes. Serial dilutions of the plant extracts were prepared in a 1:1 ratio by accurately weighing 1 g each of *Ficus religiosa* and *Bryophyllum pinnatum* extracts and dissolving them in 1 mL ethanol, followed by dilution with 10 mL distilled water. Further serial dilutions were prepared to obtain concentrations of 100, 50, 25, 12.5, and 6.25 mg/mL, designated as T1–T5, respectively. Each tube was inoculated with 1 mL of *Staphylococcus aureus* suspension. Control tubes (broth control and solvent control) were maintained. All tubes were incubated at 37°C for 18–24 hours. Tubes were examined for turbidity after incubation. The lowest concentration showing no visible growth.[14]

Fig 9: MIC Determination by Broth Dilution Method.

2.8 Antimicrobial assay of Plant extracts (Agar-well diffusion method)

2.8.1 Agar Preparation: 1.4 grams of Nutrient agar powder was dissolved in 50 ml of distilled water. The solution was sterilized using an autoclave at 121°C for 20 minutes.

2.8.2 Antimicrobial Activity of Combined Extract (Agar Well Diffusion Method)

The antimicrobial activity of the combined extract of *Ficus religiosa* and *Bryophyllum pinnatum* was evaluated using the agar well diffusion method against *Staphylococcus aureus*. Sterile nutrient agar plates were prepared and allowed to solidify under aseptic conditions. *S. aureus* was uniformly spread using 0.1 mL inoculum and a sterile spreader. A sterile cork borer was used to aseptically bore 6 mm-diameter wells into the agar. Combined extracts were made at concentrations of 2.5% and 5% based on MIC values. Each concentration was added to the corresponding wells at an amount of 100 µL (0.1 mL). For one to two hours, plates were maintained at 4°C to ensure that the extracts properly diffused into the agar. After that, the plates were incubated for 24 hours at 37°C. To evaluate antibacterial activity, the zones of inhibition (mm) were measured following incubation.[15]

Calculation of % Activity

$$\text{Activity (\%)} = \frac{\text{Zone of inhibition of sample}}{\text{Zone of inhibition of standard}} \times 100$$

Fig 10: Incubation of Agar Plates During Antimicrobial Evaluation.

2.9 Procedure for *Ficus religiosa* and *Bryophyllum pinnatum* hydrogel formulation

2.9.1 Procedure

The polyherbal hydrogel was prepared by first accurately weighing the required quantities of sodium alginate and carboxymethyl cellulose (CMC), which were then dissolved in distilled water under continuous stirring to obtain a uniform and homogeneous polymer solution. These polymers served as the structural backbone of the hydrogel and contributed to its viscosity and gel-forming properties. Once the polymer solution was prepared, ammonium persulfate (APS) was added as an initiator and mixed thoroughly to ensure its uniform distribution throughout the formulation. Subsequently, N,N′-methylene bisacrylamide (MBA) was incorporated as a cross-linking agent to facilitate the formation of a stable three-dimensional hydrogel network, thereby enhancing the mechanical strength and stability of the formulation. Glycerol was then added to improve the flexibility, softness, and moisture-retaining capacity of the hydrogel. To prevent microbial contamination and improve the shelf life of the product, methyl paraben and propyl paraben were incorporated as preservatives. Additionally, 1–2 drops of clove oil were added to provide antimicrobial properties and contribute to the overall therapeutic potential of the formulation. The mixture was continuously stirred until a smooth and homogeneous gel base was obtained and then allowed to stand for sufficient time to ensure proper gel formation. Following the preparation of the gel base, optimized quantities of *Ficus religiosa* and *Bryophyllum pinnatum* extracts were incorporated gradually with continuous stirring to ensure uniform distribution of the active constituents throughout the hydrogel matrix. The final formulation was mixed thoroughly at room temperature until a consistent gel-like texture was achieved. The prepared polyherbal hydrogel was then transferred into sterilized containers, properly labeled, and stored in a cool and dry place until further evaluation and use.[16]

Fig 11: In-house Hydrogel formulation.

2.9.2 Formulation of batches of hydrogel

Four batches of Hydrogel were prepared using the same base formulation with varying concentration of *Ficus religiosa* and *Bryophyllum pinnatum* extracts.

Table 2: Optimization Batches of Polyherbal Hydrogel Formulation.

Ingredients	Batch A	Batch B	Batch C	Batch D	Role
CMC	0.20g	0.30g	0.40g	0.50g	Gelling agent
Sodium alginate	0.05g	0.10g	0.15g	0.20g	Thickener
APS	0.002g	0.002g	0.002g	0.002g	Initiator
MBA	0.001g	0.001g	0.001g	0.001g	Cross-linker
Glycerol	2ml	2ml	2ml	2ml	Plasticizer
Plant extracts	0.50g (2.5%)	0.50g (2.5%)	1.0g (5%)	1.0g (5%)	API
Methyl paraben	0.15g	0.15g	0.15g	0.15g	Preservative
Propyl paraben	0.30g	0.30g	0.30g	0.30g	Preservative
Clove oil	1-2 drops	1-2 drops	1-2 drop	1-2 drops	Bactericidal agent
Sandalwood	2-3 drops	2-3 drops	2-3 drop	2-3 drops	Fragrance
Distilled water	q. s. to 20 ml	q.s. to 20 ml	q.s. to 20 ml	q.s. to 20 ml	Vehicle

Four hydrogel batches (A, B, C, and D) were prepared with varying polymer concentrations for formulation optimization. Batch D was omitted from the antimicrobial assay due to excessive stiffness of the hydrogel matrix, which restricted proper diffusion of active constituents. Therefore, antimicrobial evaluation was carried out only for Batches A, B, and C.

2.9.3 Antimicrobial assay for Inhouse formulations

The antimicrobial activity of the in-house hydrogel formulations was evaluated against *Staphylococcus aureus* using the agar well diffusion method. Sterile nutrient agar plates were prepared, inoculated with 0.1 mL bacterial suspension, and wells of 6 mm diameter were bored aseptically. A fixed quantity of each hydrogel formulation from Batch A, Batch B, and Batch C was introduced into the respective wells, followed by incubation at 37°C for 24 hours, after which the zones of inhibition were measured. Batch D was omitted from antimicrobial evaluation because excessive stiffness of the hydrogel matrix restricted proper diffusion of active constituents.

2.10 Evaluation of Wound Healing Hydrogel

The formulated polyherbal hydrogel containing *Ficus religiosa* and *Bryophyllum pinnatum*

extracts was evaluated for physical characteristics, extrudability, pH, loss on drying, spreadability, and *in vitro* drug diffusion.[18]

2.10.1 Physical evaluation

The prepared hydrogel was evaluated for organoleptic characteristics including colour, odour, homogeneity, and consistency. Colour was visually inspected for uniformity and appearance, odour was determined by gentle smelling, homogeneity was checked visually for uniform distribution without lumps, and consistency was assessed by manual application for smoothness and absence of grittiness.[19]

2.10.2 Extrudability test

A collapsible tube was filled with the hydrogel and sealed. The extruded gel was collected when the full tube was placed firmly between two glass slides, 500 g of weight was applied, and the cap was taken off. Extrudability was calculated by dividing the weight of the extruded gel by the extrusion time, which was noted.[20]

2.10.3 Determination of pH

About 1 g of hydrogel was dispersed in 10 mL distilled water. Buffer solutions with pH values of 4.0, 7.0, and 9.2 were used to calibrate the pH meter. The electrode was then placed into the water mixture, and the pH was measured once it stabilized. This process was done three times, and the average pH value was calculated.[21]

2.10.4 Loss on drying

Approximately 2 g of hydrogel was accurately weighed into a petri dish and the weight was recorded. The sample was divided into two groups for stability study, with one group stored under refrigerated condition at 4 ± 2°C and the other at room temperature at 25 ± 2°C. At predetermined intervals, samples were withdrawn, dried in a hot air oven at 105°C for 12 hours or until constant weight, and the final weight was recorded to calculate loss on drying.

The Loss on Drying (LOD %) was calculated using the standard formula.

$$\text{LOD (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

$\mathbf{W_1}$ = Weight of empty container (petri dish)

$\mathbf{W_2}$ = Weight of container with sample before drying

$\mathbf{W_3}$ = Weight of container with sample after drying

2.10.5 Determination of spreadability

About 1 g of hydrogel was placed on a 10 × 10 cm glass slide and covered with another slide. A weight of 2 kg was applied, and after 30 minutes the diameter of spread was measured. This process was repeated three times, and the average values was recorded.

Calculation

$$S = \frac{M \times L}{T}$$

Where:

S= Spreadability

M= Weight applied (g)

L= Distance moved (cm)

T= Time taken (s)

2.10.6 Franz diffusion study

The *in vitro* drug diffusion study was carried out using a Franz diffusion cell fitted with an onion membrane as a natural semipermeable membrane. The receptor compartment was filled with phosphate buffer pH 7.4 and maintained at 37 ± 0.5°C with continuous magnetic stirring, while a measured quantity of hydrogel was placed in the donor compartment. Samples were withdrawn from the receptor compartment at predetermined intervals, replaced with fresh phosphate buffer to maintain sink conditions, and analyzed by UV-Visible spectrophotometry at 289 nm to determine cumulative percentage drug release.[22]

3. RESULTS AND DISCUSSION

3.1 Extraction Method with % Yield

- The crude extract of *Ficus religiosa* leaves was obtained by Soxhlet extraction method using ethanol as solvent. The extract was concentrated by evaporating the solvent on a water bath, and the percentage yield was found to be 20%.
- The crude extract of *Bryophyllum pinnatum* leaves was obtained by maceration using distilled water as solvent. The extract was concentrated by evaporating the solvent on a water bath, and the percentage yield was found to be 25%.

3.2 Phytochemical Analysis

The ethanolic extract of *Ficus religiosa* and Distilled water extract of *Bryophyllum pinnatum* leaves showed the presence of flavonoids, alkaloids, glycosides, steroids, and terpenoids.

Table 3: Phytochemical Screening Results of *F. religiosa* and *B. pinnatum* extracts.

Sr. No.	Test name	*Ficus religiosa*	*Bryophyllum pinnatum*
1.	NaOH / Alkaline Reagent Test	Positive	Positive
2.	Lead Acetate Test	Positive	Positive
3.	Pew's Test	Positive	Positive
4.	Dragendorff's Test	Positive	Positive
5.	Wagner's Test	Positive	Positive
6.	Tannic Acid	Positive	Positive
7.	Liebermann–Burchard Test	Positive	Positive
8.	Salkowski Test	Positive	Positive

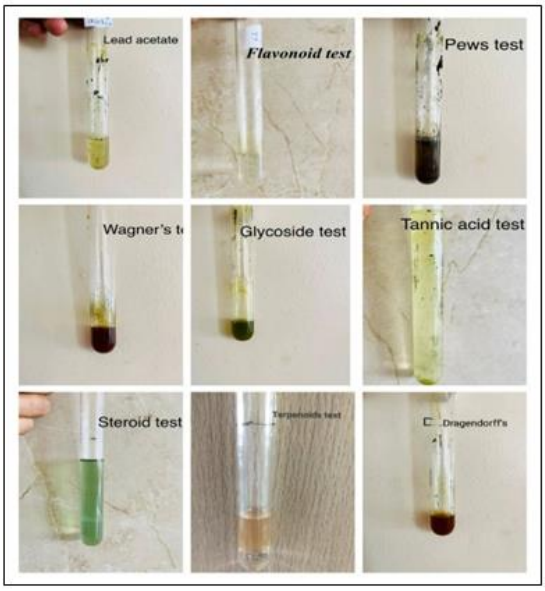

Fig 12: Preliminary Phytochemical Screening of **F. religiosa** *extract*

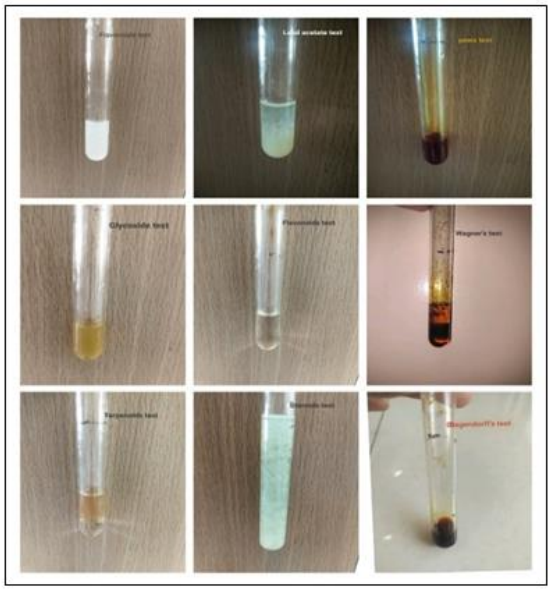

Fig 13: Preliminary Phytochemical Screening of B. pinnatum extract

3.3 Determination of Total Phenolic Content

3.3.1 Standard Calibration Curve of Gallic Acid

Table 4: Standard calibration curve data of Gallic acid for TPC determination.

Sr. No.	Concentration (µg/mL)	Absorbance (Reading 1)	Absorbance (Reading 2)	Absorbance (Reading 3)	Mean Absorbance
1.	10	0.1548	0.1560	0.1572	0.1560
2.	20	0.2315	0.2330	0.2342	0.2329
3.	30	0.2728	0.2741	0.2753	0.2741
4.	40	0.3079	0.3096	0.3112	0.3096
5.	50	0.3928	0.3946	0.3963	0.3946

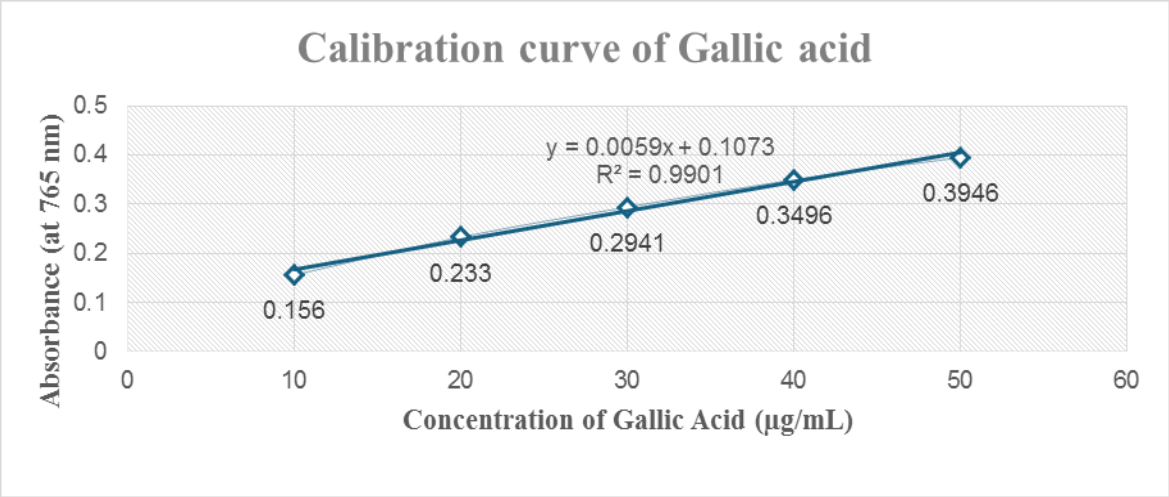

Fig 14: Standard Calibration Curve of Gallic Acid.

The calibration curve showed good linearity with an R^2 value of 0.9901, indicating a reliable relationship between concentration and absorbance for phenolic quantification.

Table 5: TPC estimation of *Ficus religiosa* extract using the Folin–Ciocalteu method.

Sr. No.	Concentration (µg/mL)	Absorbance (Reading 1)	Absorbance (Reading 2)	Absorbance (Reading 3)	Mean Absorbance	TPC (mg GAE/g)
1	10	0.368	0.372	0.369	0.3699	44.51
2	20	0.418	0.421	0.420	0.4196	52.92
3	30	0.423	0.426	0.425	0.4247	53.78
4	40	0.490	0.495	0.492	0.4924	65.27
5	50	0.536	0.541	0.540	0.539	73.17

3.3.2 Total Phenolic Content for *Ficus religiosa* Extract

TPC increased progressively with concentration from 44.51–73.17 mg GAE/g, suggesting a concentration-dependent increase in phenolic compounds.

3.3.3 Total Phenolic Content for *Bryophyllum pinnatum* Extract

Table 6: TPC estimation of *Bryophyllum pinnatum* extract using the Folin–Ciocalteu method.

Sr. No.	Concentration (µg/mL)	Absorbance (Reading 1)	Absorbance (Reading 2)	Absorbance (Reading 3)	Mean Absorbance	TPC (mg GAE/g)
1	20	0.000	0.000	0.000	0.000	0
2	40	0.037	0.036	0.037	0.0367	0
3	60	0.083	0.081	0.082	0.0820	0
4	80	0.147	0.144	0.146	0.1456	6.49
5	100	0.233	0.230	0.232	0.2317	21.08

Bryophyllum pinnatum extract showed comparatively lower total phenolic content, with TPC

values increasing gradually at higher concentrations. The observed increase suggests the presence of phenolic compounds that may contribute to its biological and antioxidant activities.

Table 7: Absorbance readings and TPC values of combined plant extracts at different concentrations.

Sr. No.	Concentration (µg/mL)	Absorbance (Reading 1)	Absorbance (Reading 2)	Absorbance (Reading 3)	Mean Absorbance	TPC (mg GAE/g)
1	20	0.244	0.247	0.246	0.246	23.51
2	40	0.303	0.307	0.305	0.305	33.51
3	60	0.363	0.367	0.365	0.365	43.68
4	80	0.392	0.396	0.394	0.394	48.59
5	100	0.421	0.426	0.424	0.424	53.68

3.3.4 Total Phenolic Content for Combined Extract.

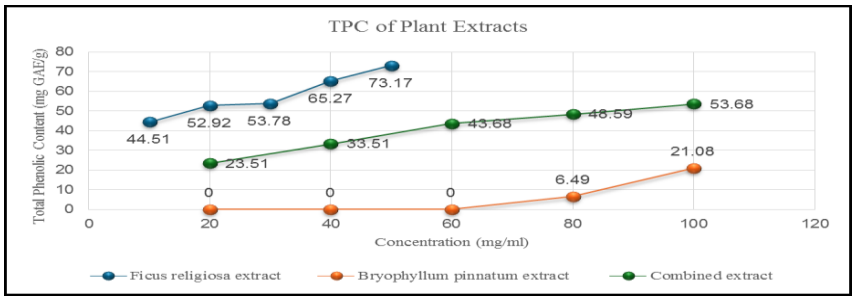

Fig 15: Comparative Analysis of Total Phenolic Content of Plant Extracts.

Ficus religiosa exhibited the highest TPC (44.51–73.17 mg GAE/g), indicating a rich phenolic profile. *Bryophyllum pinnatum* showed comparatively low TPC, with negligible values at lower concentrations and a maximum of 21.08 mg GAE/g. The combined extract demonstrated intermediate TPC values (23.51–53.68 mg GAE/g), confirming the contribution of both plant extracts. The increase in TPC in the combined extract compared to *Bryophyllum pinnatum* alone suggests a synergistic effect, supporting the rationale for selecting the combination.

3.4 Calculation of Rf values

The Rf values obtained from TLC analysis were 0.69 for *Ficus religiosa* and 0.57 for *Bryophyllum pinnatum*, calculated as the ratio of the distance travelled by the solute to that travelled by the solvent front.

3.5 Minimum Inhibitory Concentration of Plant Extracts

Table 8: MIC of combined Plant Extracts against *Staphylococcus aureus.*

Test Tube no.	Concentration (mg/ml)	Visual Observation	Bacterial growth
T1	100	Clear	No
T2	50	Clear	No
T3	25	Clear	No
T4	12.5	Turbid	Yes
T5	6.25	Turbid	Yes

Fig 16: Results of MIC.

Therefore, the minimum inhibitory concentration (MIC) of the combined extract against *S. aureus* was found to be 25 mg/mL, as it was the lowest concentration with no visible growth.

3.6 Antimicrobial assay for Combined Plant extracts

Table 9: Antimicrobial activity of combined plant extracts compared to standard.

Sample	Zone of inhibition (in mm)	Activity (%) relative to standard
Standard (Ciprofloxacin)	65 mm	100%
Sample 1 (2.5%)	35mm	53.85%
Sample 2 (5%)	37.5 mm	57.69%

The standard drug ciprofloxacin produced a 65 mm zone of inhibition, confirming the validity of the method. The combined extract exhibited concentration-dependent antimicrobial activity, showing zones of inhibition of 35 mm at 2.5% concentration and 37.5 mm at 5% concentration. The increase in concentration resulted in enhanced antibacterial activity. Although the activity was lower than that of the standard, the combined extract demonstrated moderate antimicrobial potential, which may be attributed to the presence of phenolic and flavonoid compounds. The results also suggest a possible synergistic effect between the extracts, supporting their combined use.

Fig 17: (A) Zone of inhibition of Standard. (B) Zone of inhibition of Combined extract.

3.7 Formulation Optimization

Four hydrogel batches, namely Batch A, Batch B, Batch C, and Batch D, were prepared using the same base formulation with variation in polymer and extract concentrations for optimization of the formulation. Batch D was excluded from the antimicrobial assay because the hydrogel matrix was excessively stiff and did not permit proper diffusion of the active constituents.

3.7.1 Anti-microbial activity of hydrogel

The hydrogel formulations demonstrated significant antimicrobial activity against *Staphylococcus aureus*. Among all batches, Batch B showed the highest activity with a zone of inhibition of 49 mm and activity relative to standard of 75.38, followed by Batch C with 42 mm and 64.61, and Batch A with 37 mm and 56.92.

The enhanced activity of Batch B may be due to optimum polymer and extract concentration, allowing better diffusion of active constituents. Hence, Batch B was selected as the optimized formulation and subjected to further evaluation studies.

Table 10: Antimicrobial Activity of Hydrogel Formulation Against Staphylococcus aureus.

Sample	Zone of Inhibition (mm)	Activity (%) Relative to Standard
Standard (Ciprofloxacin)	65 mm	100%
Batch A	37 mm	56.92%
Batch B	49 mm	75.38%
Batch C	42 mm	64.61%

Fig 18: Zones of Inhibition Produced by Hydrogel Formulations (Batch A, B, C).

The hydrogel formulations exhibited notable antimicrobial activity, with Batch B showing the highest zone of inhibition (49 mm, 75.38%), followed by Batch C (42 mm, 64.61%) and Batch A (37 mm, 56.92%). The enhanced activity of Batch B may be attributed to the optimal concentration of polymer and herbal extracts, facilitating better release of active constituents. Therefore, Batch B was selected as the optimized formulation for further studies.

3.8 Evaluation of Hydrogel Formulation

3.8.1 Physical Evaluation

The formulated hydrogel exhibited a pale yellow to light beige colour with a translucent appearance. The formulation showed smooth, uniform, and semi-solid consistency without coarse particles, indicating good homogeneity.

3.8.2 Extrudability

The formulation exhibited satisfactory extrudability with a value of approximately 1.4 g/s, indicating ease of extrusion from the collapsible tube and convenient topical application.

Fig 19: Packed and Labelled Formulation.

3.8.3 pH Determination

The pH of the hydrogel was found to be **6.36**, which is within the acceptable range for topical formulations and compatible with skin pH, thereby minimizing the chances of skin irritation.

3.8.4 Loss on Drying (LOD)

The optimized hydrogel formulation demonstrated better moisture retention and stability under refrigerated conditions compared to room temperature storage. Under refrigerated condition, the recorded values after drying were 51.20 g on Day 0, 51.25 g on Day 4, and 51.30 g on Day 7, with corresponding LOD values of 40, 37.5, and 35, respectively.

At room temperature, the recorded values after drying were 51.20 g on Day 0, 51.10 g on Day 4, and 51.00 g on Day 7, with corresponding LOD values of 40, 45, and 50, respectively.

Table 11: Loss on Drying (LOD) Study of Hydrogel Under Refrigerated Conditions (4°C).

Day	W_3 (After Drying)	LOD (%)	Observation
Day 0	51.20 g	40%	Initial
Day 4	51.25 g	37.5%	Slight moisture retention
Day 7	51.30 g	35%	Stable

Table 12: Loss on Drying (LOD) Study of Hydrogel Under Room Temperature Conditions (25–35°C).

Day	W_3 (After Drying)	LOD (%)	Observation
Day 0	51.20 g	40%	Initial
Day 4	51.10 g	45%	Slight moisture loss
Day 7	51.00 g	50%	Increased drying

The optimized hydrogel formulation demonstrated better moisture retention and stability under refrigerated conditions compared to room temperature storage.

3.8.5 Spreadability

The hydrogel showed good spreadability with a calculated value of approximately **320 g·cm/s**.

3.8.6 Franz Diffusion Study

The *in vitro* drug diffusion study of the formulated polyherbal hydrogel was carried out using a Franz diffusion cell with phosphate buffer pH 7.4 as the receptor medium. The study demonstrated a gradual and sustained release of the active constituents from the hydrogel matrix over the study period.

The percentage drug release increased progressively with time, showing 18 at 15 minutes, 36 at 30 minutes, 58 at 45 minutes, and 75 at 60 minutes. The increase in drug release with time

indicates effective diffusion of phytoconstituents through the dialysis membrane from the hydrogel system.

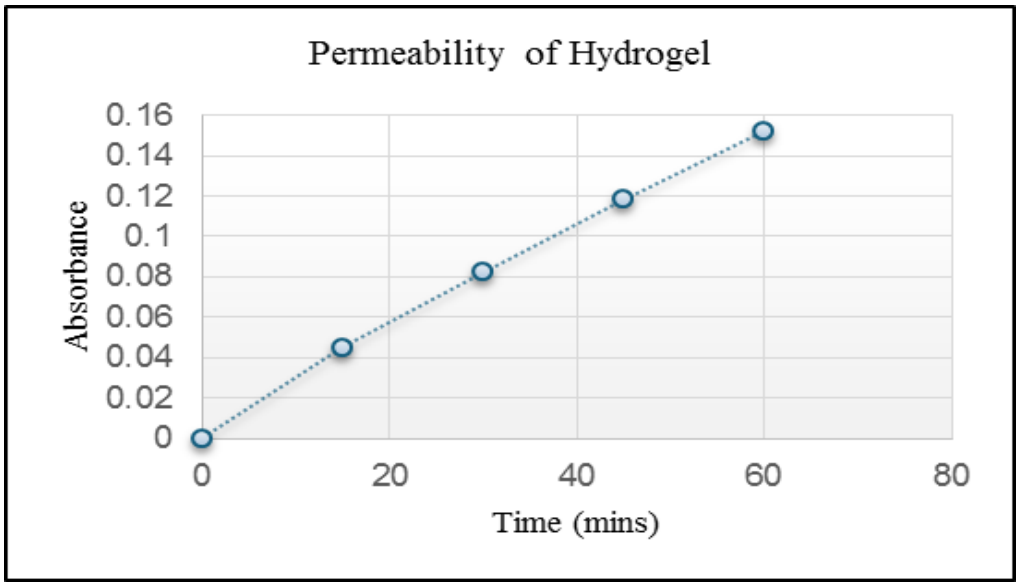

Fig 20: Cumulative Drug Release of Hydrogel Over Time.

Table 13: Drug Diffusion Profile of Hydrogel Formulation.

Time (min)	Absorbance	% Drug Release
0	0.000	0%
15	0.045	18%
30	0.082	36%
45	0.118	58%
60	0.152	75%

The sustained release behaviour may be attributed to the three-dimensional polymeric network formed by sodium alginate and CMC, which controls the movement of active constituents and prevents rapid drug leakage. The presence of glycerol also contributed to maintaining hydration and flexibility of the gel matrix, thereby supporting controlled diffusion.

The release profile suggested a diffusion-controlled mechanism and was found to follow Higuchi kinetics, indicating that the drug release occurred mainly through diffusion from the hydrated polymer matrix. Such sustained release behaviour is advantageous for topical wound healing formulations as it helps maintain prolonged contact of the active constituents at the wound site, reduces the frequency of application, and improves therapeutic efficacy.

Overall, the Franz diffusion study confirmed that the formulated hydrogel had suitable release characteristics for effective topical drug delivery.

4. CONCLUSION

The present study focused on the formulation and evaluation of a polyherbal hydrogel containing extracts of *Ficus religiosa* and *Bryophyllum pinnatum* for wound healing and antimicrobial applications. Phytochemical screening confirmed the presence of bioactive constituents such as flavonoids, phenolics, alkaloids, terpenoids, and glycosides, which contribute to antioxidant, antimicrobial, and tissue regenerative activities. The combined extract demonstrated enhanced bioactive potential, while TLC analysis confirmed the presence of important phytoconstituents. The increase in TPC suggests a possible synergistic interaction, supporting the rationale for the combination The formulated hydrogel exhibited satisfactory physicochemical properties, including appropriate pH, good spreadability, acceptable extrudability, and stability under refrigerated conditions. Antimicrobial studies revealed concentration-dependent activity, with Batch B showing the highest antibacterial efficacy and therefore identified as the optimized formulation. The Franz diffusion study indicated sustained and diffusion-controlled release of active constituents from the hydrogel matrix. Overall, the developed polyherbal hydrogel demonstrated promising potential as a natural topical formulation for antimicrobial and wound care applications.

5. FUTURE SCOPE

Further studies are required to establish its therapeutic effectiveness and clinical applicability. Future research may focus on *in vivo* wound healing studies, histopathological investigations, and advanced characterization techniques such as FTIR, SEM, and rheological analysis to better understand the formulation's structural and functional properties. Long-term stability studies under various environmental conditions should also be conducted to determine shelf life and storage requirements. Additionally, incorporation of advanced drug delivery approaches, including nanoparticles, nanoemulsions, or liposomes, may enhance the penetration and controlled release of bioactive compounds. Evaluation against a broader spectrum of wound-associated microorganisms, including multidrug-resistant strains, along with clinical studies and large-scale production strategies, may further support the development of this formulation as a safe, effective, economical, and herbal alternative for wound care management.

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academic environment throughout the project work.

7. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research work.

8. REFERENCES

1. Almadani, Y. H., Vorstenbosch, J., Davison, P. G., & Murphy, A. M. Wound Healing: A Comprehensive review. *Seminars in Plastic Surgery*, 2021; *35*(03): 141–144. https://doi.org/10.1055/s-0041-1731791
2. Cedillo-Cortezano, M., Martinez-Cuevas, L. R., Márquez López, J. A., Barrera López, I. L., Escutia-Perez, S., & Petricevich, V. L. *Use of medicinal plants in the process of wound healing: A literature review*. *Pharmaceuticals*, 2024; *17*(3): 303. https://doi.org/10.3390/ph17030303
3. Kapile, C., Kulkarni, A., Pardeshi, P., Sayed, A., & Nehe, A. *Ficus religiosa*: A beneficial medicinal plant. *Journal of Drug Delivery and Therapeutics*, 2022; *12*(2-S): 210–218. https://doi.org/10.22270/jddt.v12i2-s.5434
4. Sharma, G., Jangra, A., Sihag, S., Chaturvedi, S., Yadav, S., & Chhokar, V. *Bryophyllum pinnatum* (Lam.) Oken: unravelling therapeutic potential and navigating toxicity. *Physiology and Molecular Biology of Plants*, 2024; *30*(9): 1413–1427. *https://doi.org/10.1007/s12298-024-01509-7*
5. Esmaiel Jabbari & Filippo Rossi (Eds.). Advances in Hydrogel-Based drug delivery systems. In *Gels*, 2024; 10: 262. https://doi.org/10.3390/gels10040262
6. [1] Kaur, H., ‡, Gogoi, B., ‡, Sharma, I., Das, D. K., Azad, M. A., Pramanik, D. D., & Pramanik, A. Hydrogels as a potential biomaterial for multimodal therapeutic applications. *Mol. Pharmaceutics*, 2024; 4827–4848. https://doi.org/10.1021/acs.molpharmaceut.4c00595
7. A comprehensive review: - Phytochemical screening and extraction of ficus Religiosa Linn. *IJNRD - International Journal of Novel Research and Development*, 2025; *10*(11): a706–a710. https://www.ijnrd.org
8. Gaikwad, S., Gaikwad, S., Gagre, R., Desai, P., Patil, P., & Khadre, V. Extensive study of formulation and *Bryophyllum pinnatum* plant. *International Journal of Pharmaceutical Sciences*, 2025; *3*(4): 483–491. https://doi.org/10.5281/zenodo.15142092
9. Gregory, M., Divya, B., Mary, R. A., Viji, M. H., Kalaichelvan, V., & Palanivel, V. Anti–

ulcer activity of Ficus religiosa leaf ethanolic extract. *Asian Pacific Journal of Tropical Biomedicine*, 2013; *3*(7): 554–556. https://doi.org/10.1016/s2221-1691(13)60112-4

10. M, S., Shah, M., & Pande, M. Determination of Total Phenols and Flavonoid Content of Bryonia laciniosa by Spectrophotometric Method. *Journal of Chemical and Pharmaceutical Research*, 2018; 30–34.
11. Kamtekar, S., et al., Keer, V., Patil, V., & M.E.T Institute of Pharmacy. Estimation of Phenolic content, Flavonoid content, Antioxidant and Alpha amylase Inhibitory Activity of Marketed Polyherbal Formulation. In *Journal of Applied Pharmaceutical Science*, 2014; 4–09: 061–065, [Journal-article]. https://doi.org/10.7324/JAPS.2014.40911
12. Jaafar, N. S., Hamad, M. N., Alshammaa, D. A., & Noori, Z. S. Phytochemical study and thin layer chromatography of Ficusreligiosa leaves extract cultivated in Iraq. *Al Mustansiriyah Journal of Pharmaceutical Sciences*, 2021; *21*(2).
13. Araújo, E. R. D., Xavier-Santos, J. B., Da Silva, V. C., De Lima, J. B. F., Schlamb, J., De Freitas Fernandes-Pedrosa, M., Da Silva Júnior, A. A., De Araújo Júnior, R. F., Rathinasabapathy, T., Moncada, M., Esposito, D., 1Guerra, G. C. B., & Zucolotto, S. M. Gel formulated with Bryophyllum pinnatum leaf extract promotes skin wound healing in vivo by increasing VEGF expression: A novel potential active ingredient for pharmaceuticals. *Frontiers in Pharmacology*, 2023; *13:* 1104705. https://doi.org/10.3389/fphar.2022.1104705
14. Paray, A. A., Singh, M., & Mir, M. A. Gram Staining: A Brief review. *International Journal of Research and Review*, 2023; *10*(9): 336–341. https://doi.org/10.52403/ijrr.20230934
15. Hulankova, R. Methods for determination of antimicrobial activity of essential oils in Vitro—A Review. In Milena D Vuki´c & Andrea Maxia (Eds.), *Plants*, 2024; 13: 2784 [Journal-article]. https://doi.org/10.3390/plants13192784
16. Hossain, T. J. Methods for screening and evaluation of antimicrobial activity: A review of protocols, advantages, and limitations. *European Journal of Microbiology and Immunology*, 2024; *14*(2): 97–115. https://doi.org/10.1556/1886.2024.00035
17. Manjula, B., Varaprasad, K., Sadiku, R., & Raju, K. M. Preparation and characterization of Sodium Alginate–Based Hydrogels and their in vitro release studies. *Advances in Polymer Technology*, 2013; *32*(2). https://doi.org/10.1002/adv.21340
18. Patil, S., Bichave, A., Deshmukh, M., & Tilak, P. Formulation of herbal soap with potential antibacterial activity. *Indian Research Journal of Pharmacy and Science*, 2021; 8(4): 2638–2643.

19. Ahmed EM. Hydrogel: Preparation, characterization and applications. *Journal of Advanced Research*. 2015; 6(2): 105-121.
20. Boateng JS, Matthews KH, Stevens HNE, Eccleston GM. Wound healing dressings and drug delivery systems: A review. *Journal of Pharmaceutical Sciences*. 2008; 97(8): 2892-2923.
21. Peppas NA, Bures P, Leobandung W, Ichikawa H. Hydrogels in pharmaceutical formulations. *European Journal of Pharmaceutics and Biopharmaceutics*. 2000; 50(1): 27-46.
22. Franz TJ. Percutaneous absorption: On the relevance of in vitro data. Journal of Investigative Dermatology. 1975; 64(3): 190-195.