**Journal of Neonatal Surgery**
***ISSN(Online): 2226-0439***
***Vol. 14, Issue 31s (2025)***
*https://www.jneonatalsurg.com*

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# Phytotherapeutic Potential Of Bauhinia Racemosa In A Topical Lotion For Bacterial Infections

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Cite this paper as: Virendra Singh Latala, Nitin Nama, M. K. Gupta, Girish Kumar Vyas, (2025) Phytotherapeutic Potential Of Bauhinia Racemosa In A Topical Lotion For Bacterial Infections. *Journal of Neonatal Surgery*, 14 (31s), 101-111.

## ABSTRACT

**Introduction**: Bacterial skin infections remain a public health challenge, particularly with the rise in antibiotic resistance. Traditional medicinal plants like *Bauhinia racemosa* have shown promise due to their phytochemical-rich profile and ethnomedicinal relevance.

**Objective**: This study aimed to formulate and evaluate a topical lotion containing *Bauhinia racemosa* leaf extract for its antibacterial potential and physiochemical stability.

**Methods**: Dried *Bauhinia racemosa* leaves were extracted using ethanol in a Soxhlet apparatus. Phytochemical screening identified flavonoids, tannins, alkaloids, saponins, and terpenoids. Characterization was done using TLC and UV-Visible spectroscopy. The extract was formulated into an oil-in-water lotion and evaluated for pH, viscosity, spreadability, homogeneity, and stability over 90 days. Antibacterial activity was tested using agar well diffusion, disc diffusion, and broth dilution (MIC) methods against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

**Results**: The lotion demonstrated effective antibacterial activity, especially against *S. aureus*, and retained favorable pH (4.5–6.8), viscosity, and appearance over 90 days. No phase separation or microbial contamination was observed.

**Conclusion**: The topical formulation of *Bauhinia racemosa* shows promising antibacterial efficacy and stability, supporting its use as a cost-effective, natural alternative for managing skin infections.

***Keywords:*** *Bauhinia racemosa, antibacterial lotion, phytochemicals, topical formulation, skin infection.*

## 1. INTRODUCTION

Bacterial infections continue to pose significant public health challenges, necessitating the pursuit of alternative, cost-effective treatments. Among promising candidates, traditional medicinal plants have garnered considerable attention for their bioactive compounds with inherent antibacterial properties1. Bauhinia racemosa, a small tree widely distributed in tropical regions, holds a prominent place in ethnomedicine for treating various skin disorders and microbial infections. Its phytoconstituents—including flavonoids, tannins, and phenolics are reported to exhibit potent antibacterial activity2,3. This study investigates the phytotherapeutic potential of Bauhinia racemosa by formulating a topical lotion and evaluating its antibacterial efficacy. The research focuses on standardizing extract preparation, conducting phytochemical screening, and developing a stable, skin-compatible formulation. By aligning traditional knowledge with modern pharmaceutical approaches, this study aims to explore Bauhinia racemosa as a natural, accessible, and effective antibacterial agent. The findings are expected to contribute to the growing interest in plant-based therapeutics, offering new avenues for affordable and sustainable skincare solutions2-4.

## 2. OBJECTIVE OF THE STUDY

The objective of this study is to formulate and evaluate a topical lotion containing Bauhinia racemosa extract, assessing its antibacterial activity through in vitro models. It also aims to identify active phytochemicals and explore the formulation's cost-effectiveness compared to conventional therapies.

## 3. MATERIALS AND METHODS

The Materials and Methods section is a crucial component of this study, enabling reproducibility, transparency, and validation of the research outcomes. It outlines the specific materials used, along with the detailed methodologies employed for extraction, phytochemical screening, characterization, and formulation.

### Materials

**1. Plant Material: Bauhinia racemosa**: Dried and powdered leaves were collected and stored in airtight containers until use.

**2. Solvents and Reagents:** Ethanol or Methanol (analytical grade), Distilled water, Mayer's, Wagner's, and Dragendorff's reagents, Ferric chloride solution, Lead acetate solution, Liebermann–Burchard reagent, Shinoda reagent (magnesium + hydrochloric acid), Chloroform, sulfuric acid, Sodium hydroxide, Iodine chamber (for TLC visualization), Potassium bromide (KBr, for FTIR).

**3. Formulation Ingredients:** Stearic acid, Cetyl alcohol, Glycerin, Methylparaben, Propylparaben, Distilled water.

**4. Microbiological Materials:** Bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa,* Mueller-Hinton Agar/Nutrient Agar, Agar well borer or disc diffusion discs, Sterile Petri plates, inoculating loop, Nutrient broth, Sterile syringes and micropipettes.

**5. Instruments and Equipment:** Soxhlet apparatus, Rotary evaporator or water bath, Desiccator, UV-Visible spectrophotometer, FTIR spectrophotometer, TLC chamber and silica gel plates, Heating mantle, Magnetic stirrer or homogenizer, Brookfield viscometer, pH meter, Incubator, Glass slides.

### Methods

**1. Extraction Procedure**

Dried and powdered Bauhinia racemosa leaves (50–100 g) were placed in a thimble and extracted using a Soxhlet extractor with ethanol or methanol as the solvent. The process was carried out for 6–8 hours until the solvent in the siphon tube became colorless, indicating exhaustive extraction. The extract was then filtered, and the filtrate was concentrated using a rotary evaporator under reduced pressure. The concentrated extract was stored in a desiccator until further use[5,6].

**2. Phytochemical Screening (Qualitative Tests)**

**Alkaloids**

- **Mayer's Test**: Creamy white precipitate upon adding Mayer's reagent.
- **Wagner's Test**: Reddish-brown precipitate on adding Wagner's reagent.
- **Dragendorff's Test**: Orange-brown precipitate after reagent addition.

**Flavonoids**

- **Shinoda Test**: Addition of magnesium metal and HCl to the extract producing a pink/red coloration indicates flavonoids[6].

**Tannins/Phenols:** Ferric chloride reagent was added. A blue-black or greenish color confirms presence.

**Saponins:** The extract was shaken vigorously with water. Persistent frothing indicated the presence of saponins.

**Steroids/Terpenoids: Liebermann–Burchard Test**: Addition of acetic anhydride and concentrated $H_2SO_4$ produced a green color if steroids or terpenoids were present.

**Glycosides and Anthraquinones:** Sodium hydroxide was added to the extract. A yellow, brown, or reddish coloration indicated glycosides or anthraquinones[7].

**Table 1: Phytochemical Screening of extract[6,7]**

| S. No. | Compound Class | Test | Observation |
|---|---|---|---|
| 1 | Alkaloids | Mayer's/Wagner's/Dragendorff's | Precipitate formation |
| 2 | Flavonoids | Shinoda | Pink/red coloration |
| 3 | Tannins/Phenols | Ferric chloride | Blue-black/green color |
| 4 | Saponins | Water froth test | Persistent foam |

| | | | |
|---|---|---|---|
| 5 | Steroids/Terpenoids | Liebermann–Burchard | Green color |
| 6 | Glycosides/Anthraquinones | NaOH test | Color change |

**3. Characterization of Active Compounds**

**Thin Layer Chromatography (TLC):** A small amount of extract was spotted on a silica gel-coated TLC plate. The plate was placed in a chamber with a suitable mobile phase and developed. After drying, spots were visualized under UV light (254/365 nm) or in an iodine chamber. Retention factor (Rf) values were calculated and used for compound identification[8].

**UV-Visible Spectroscopy:** The extract was dissolved in methanol and analyzed using a UV-Vis spectrophotometer across 200–800 nm. Absorption maxima were recorded, and spectra were analyzed for peak values indicating presence of flavonoids, phenols, or alkaloids. Data were compared with literature values for compound identification[9].

**4. Formulation of Topical Lotion**

- Oil Phase: Stearic acid, cetyl alcohol, and parabens were heated to 70°C.
- Aqueous Phase: Glycerin and distilled water were separately heated to the same temperature[10].

Both phases were combined with continuous stirring using a magnetic stirrer or homogenizer until a uniform emulsion was formed. The plant extract was incorporated during the emulsification step. Optional fragrance was added after cooling. The lotion was stored in sterilized containers for further evaluation[11].

**Characterization of Lotion (Bauhinia racemosa Topical Lotion)**

- **pH Measurement**: Use a digital pH meter to measure pH and ensure it falls between 4–7 for skin compatibility.
- **Viscosity**: Measure using a Brookfield viscometer to assess lotion thickness and flow behavior.
- **Spreadability**: Place lotion between two glass slides; apply a known weight and measure the diameter of spread.
- **Homogeneity**: Visually inspect for uniform appearance, absence of clumps, or phase separation.
- **Stability Testing**: Store samples at RT, 4°C, and 40°C; observe for changes in color, odor, separation, or microbial growth over 1–3 months[10,11].

**Antibacterial Activity Assessment (In Vitro)**

- **Agar Well Diffusion Methos:** Inoculate MHA plates with bacterial strains. Punch wells (6–8 mm), add 100 µL test sample, incubate at 37°C for 24 hrs. Measure zone of inhibition[12].
- **Disc Diffusion Method**: Soak sterile filter paper discs with the sample, place on inoculated MHA plates, incubate at 37°C for 24 hrs. Measure inhibition zones[12].
- **Broth Dilution Method (MIC)**: Prepare serial dilutions of extract in broth, inoculate with bacteria, incubate at 37°C for 18–24 hrs. MIC = lowest concentration showing no turbidity[13].

**Stability Study (90 Days):** Store Formulation F3 at RT, 4°C, and 40°C. Evaluate physical appearance, pH, viscosity, homogeneity, and microbial growth on Day 0, 30, 60, and 90.[14]

**4. RESULTS**

- **Plant Collection:** Fresh *Bauhinia racemosa* leaves were collected from Career Point University, Alaniya, Kota (25.0892° N, 75.8716° E).
- **Extraction of *Bauhinia racemosa* leaves:** Ethanolic extraction of *Bauhinia racemosa* leaves (75 g) using Soxhlet yielded 3.28 g crude extract (4.37% w/w). The dark brown semi-solid with characteristic odor was stored in a desiccator for phytochemical analysis and formulation..

**Figure 1: Extraction of dried *Bauhinia racemosa* leaves**

**Table 2: Extraction results of *Bauhinia racemosa* leaves**

| Sr. No. | Parameter | Value |
|---|---|---|
| 1 | Plant Material Used | 75 g (dried leaves) |
| 2 | Solvent Used | Ethanol |
| 3 | Extraction Method | Soxhlet Extraction |
| 4 | Duration of Extraction | 6–8 hours |
| 5 | Amount of Extract Obtained | 3.28 g |
| 6 | Extraction Yield | 4.37% w/w |

- **Phytochemical Screening (Qualitative Tests):** Phytochemical screening is a crucial process for identifying the bioactive compounds present in plant extracts, which may have therapeutic potential.

**Figure 2: Phytochemical Screening of extract of *Bauhinia racemosa* leaves**

In this study, the qualitative tests for various phytochemicals in *Bauhinia racemosa* leaves were conducted to detect the presence of alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, glycosides, and anthraquinones. The following table summarizes the results of these tests, which help determine the specific compounds responsible for the plant's medicinal properties:

**Table 2: Phytochemical Screening (Qualitative Tests) results**

| Sr. No. | Phytochemical | Present/Absent |
|---|---|---|
| 1 | Alkaloids | Present |
| 2 | Flavonoids | Present |
| 3 | Tannins/Phenols | Present |
| 4 | Saponins | Present |

| | | |
|---|---|---|
| 5 | Steroids/Terpenoids | Present |
| 6 | Glycosides | Present |
| 7 | Anthraquinones | Present |

- **Characterization of Active Compounds:** Two test in this characterisation were performed.

**Thin Layer Chromatography (TLC**): Thin Layer Chromatography (TLC) was performed on the *Bauhinia racemosa* leaf extract to separate and identify the active compounds present. The extract was applied as a spot on a silica gel-coated TLC plate and developed using a solvent mixture. After development, the plate was visualized under UV light (254 nm) to observe the separated compounds. The following results were obtained:

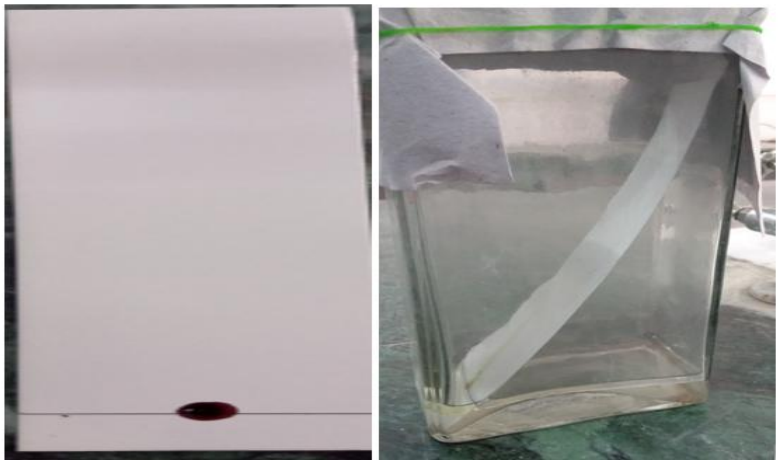

**Figure 3: TLC plate preparation and extract marking on the bottom**

**Table 4: Thin Layer Chromatography results**

| Spot No. | Rf Value | Color/Appearance | Possible Compound |
|---|---|---|---|
| 1 | 0.28 | Fluorescent green | Flavonoids |
| 2 | 0.46 | Fluorescent yellow | Alkaloids |
| 3 | 0.64 | Dark brown | Phenolic compounds |
| 4 | 0.75 | Light Orange-brown | Terpenoids |

The Rf values were calculated by measuring the distance travelled by each spot relative to the solvent front.

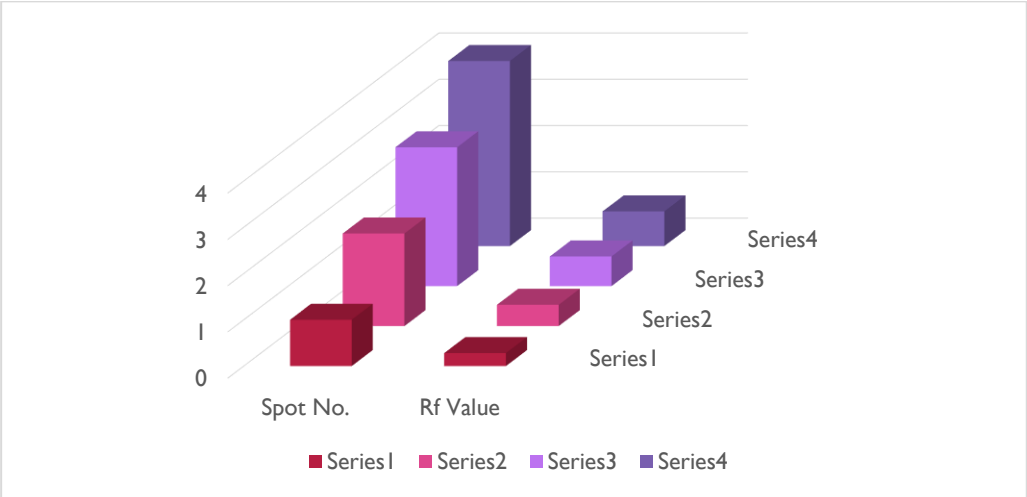


**Chart 1: Rf value determination of extract**

The compounds were identified based on their characteristic colors under UV light and their Rf values, which were compared to those of known standards.

The TLC results indicate the presence of flavonoids (Rf = 0.28), alkaloids (Rf = 0.46), phenolic compounds (Rf = 0.64), and terpenoids (Rf = 0.75) in the *Bauhinia racemosa* leaf extract, confirming the diversity of bioactive compounds in the plant.

**A. UV-Visible Spectroscopy:** UV-Visible Spectroscopy was conducted to analyze the absorption spectrum of the *Bauhinia racemosa* leaf extract. A small aliquot of the extract was dissolved in methanol and scanned across a wavelength range of 200 nm to 800 nm. The absorption spectrum revealed distinct peaks corresponding to the absorption maxima of various phytochemicals present in the extract. The following absorption peaks were observed:

**Table 5: Absorption spectrum of the *Bauhinia racemosa* leaf extract**

| Sr. No. | Wavelength (nm) | Absorbance | Possible Phytochemical |
|---|---|---|---|
| 1 | 250 nm | 0.45 | Flavonoids (characteristic peak) |
| 2 | 300 nm | 0.56 | Phenolic compounds |
| 3 | 370 nm | 0.63 | Alkaloids |
| 4 | 440 nm | 0.52 | Terpenoids |

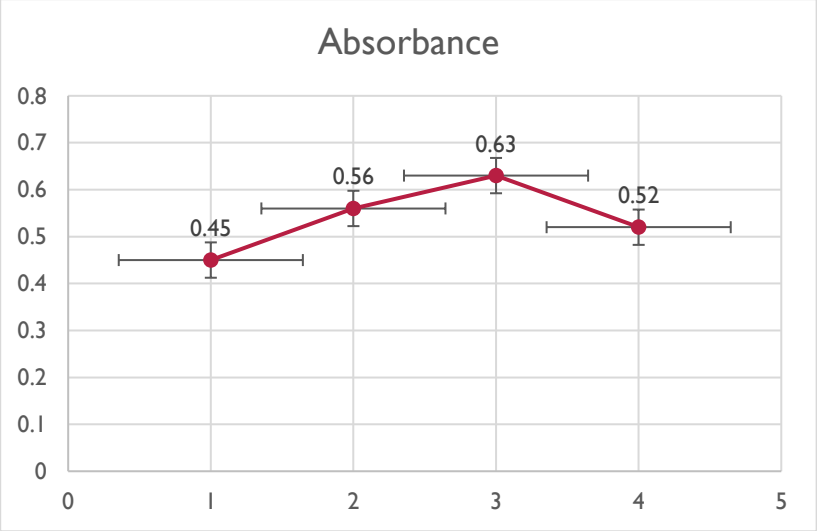


**Chart 2: Extract Absorbance at difference wavelength**

The absorption maxima at 250 nm, 300 nm, 370 nm, and 440 nm indicate the presence of flavonoids, phenolic compounds, alkaloids, and terpenoids, respectively. These peaks are consistent with the known absorption characteristics of these bioactive compounds, confirming their presence in the *Bauhinia racemosa* leaf extract.

**Figure 4: Phytochemical confirmation by UV visible spectrophotometer**

The intensity of the peaks also suggests that these compounds are present in significant quantities, which may contribute to the extract's therapeutic properties. Further analysis, such as comparison with literature values or standards, could provide more specific identification of the compounds.

- **Formulation of Topical Lotion:**

Three formulations of the topical lotion were prepared using *Bauhinia racemosa* extract with varying concentrations of the active pharmaceutical ingredient (API), which were incorporated based on their minimum inhibitory concentration (MIC) values. The following formulas were created to evaluate the antibacterial potential of the extract at different concentrations:

**Table 6: Formulations of extract with suitable excipients**

| Sr. No. | Formulation | F1 (3% API) | F2 (5% API) | F3 (10% API) |
|---|---|---|---|---|
| 1 | *Bauhinia racemosa* Extract (API) | 3 g | 5 g | 10 g |
| 2 | Stearic Acid | 5 g | 5 g | 5 g |
| 3 | Cetyl Alcohol | 3 g | 3 g | 3 g |
| 4 | Phenoxyethanol | 0.3 g | 0.3 g | 0.3 g |
| 5 | Distilled Water | 50 mL | 50 mL | 50 mL |
| 6 | Glycerin | 5 g | 5 g | 5 g |
| 7 | Fragrance | 0.5 g | 0.5 g | 0.5 g |

To formulate the topical lotion, the oil phase and aqueous phase were prepared separately before emulsification. The oil phase was prepared by heating stearic acid, cetyl alcohol, and phenoxyethanol to 70°C in a heatproof container until all ingredients were fully melted. In a separate container, the aqueous phase was prepared by heating distilled water and glycerin to the same temperature. Once both phases reached 70°C, the aqueous phase was gradually added to the oil phase under continuous stirring to form an emulsion. The mixture was stirred vigorously to ensure proper blending of both phases. After emulsification, the mixture was allowed to cool to 40°C. At this point, the *Bauhinia racemosa* extract (according to the desired concentration for each formulation) and fragrance were added, followed by homogenization to ensure uniformity and achieve a smooth, consistent texture. Finally, the lotion was transferred to sterilized containers and stored at room temperature, away from direct sunlight, with appropriate labeling for the formulation date and ingredients. This process results in a stable and effective topical lotion, utilizing the antibacterial properties of *Bauhinia racemosa*.

- **Evaluations of prepared Lotion:**

The formulated *Bauhinia racemosa* topical lotions (F1, F2, and F3) were evaluated based on various criteria to ensure their quality and suitability for skin application. The pH values of all formulations were within the skin-friendly range (4-7), indicating compatibility with the skin. Viscosity measurements using a Brookfield viscometer confirmed the desired consistency for optimal spreadability, which was assessed through the slide and weight method. All formulations demonstrated uniformity without any phase separation or grittiness during visual inspection, confirming good homogeneity. Stability tests showed that the lotions remained stable at different temperatures (room temperature, 4°C, and 40°C) over the 1–3 months observation period, with no significant physical or chemical changes. The formulations maintained their appearance, consistency, and overall effectiveness, ensuring their potential for therapeutic use.

**Table 7: Evaluation Parameters of the Prepared Formulation**

| **Sr. No.** | **Evaluation Parameter** | **Method** | **F1 (3% API)** | **F2 (5% API)** | **F3 (10% API)** |
|---|---|---|---|---|---|
| 1 | pH | Digital pH meter | 6.2 | 6.3 | 6.1 |
| 2 | Viscosity | Viscometer | 600 cps | 700 cps | 750 cps |
| 3 | Spreadability | Slide and weight method | 7.5 cm | 8 cm | 8.2 cm |

| | | | | | |
|---|---|---|---|---|---|
| 4 | Homogeneity | Visual inspection | No separation | No separation | No separation |
| 5 | Stability | Storage at different temperatures (RT, 4°C, 40°C) | Stable | Stable | Stable |

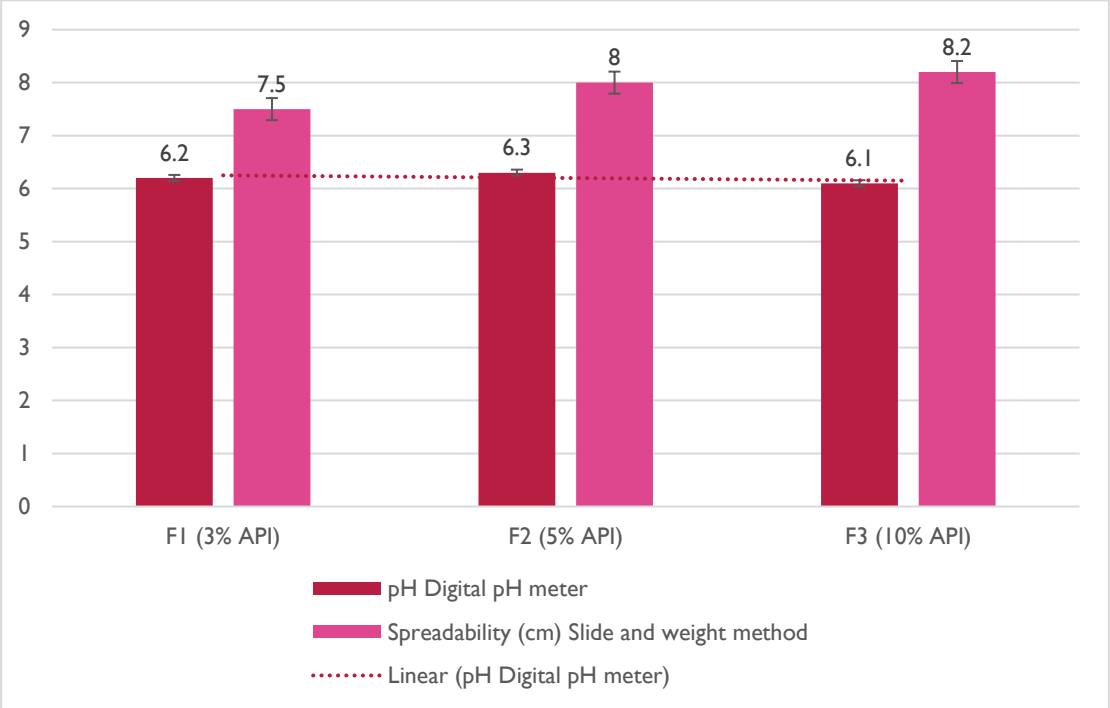


**Chart 3: pH and viscosity determination of all three formulations**

- **Antibacterial Activity Assessment of *Bauhinia racemosa* Extract and Formulated Lotions:** The antibacterial activity of *Bauhinia racemosa* extract and its topical lotion formulations (F1: 3%, F2: 5%, and F3: 10% API) was assessed through three in vitro methods: agar well diffusion, disc diffusion, and broth dilution for MIC determination.
- **Agar Well Diffusion Method:** In this method, all three formulations showed clear zones of inhibition against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, indicating broad-spectrum activity. The F3 formulation (10% API) produced the largest zones, confirming its enhanced antibacterial potency with increasing concentration. F2 and F1 followed in descending order of activity.

**Table 8: Agar Well Diffusion Results**

| **Sr. No.** | **Organism** | **Zone of Inhibition (mm) F1** |
|---|---|---|
| 1 | *Staphylococcus aureus* | 12 mm |
| 2 | *Escherichia coli* | 10 mm |
| 3 | *Pseudomonas aeruginosa* | 9 mm |

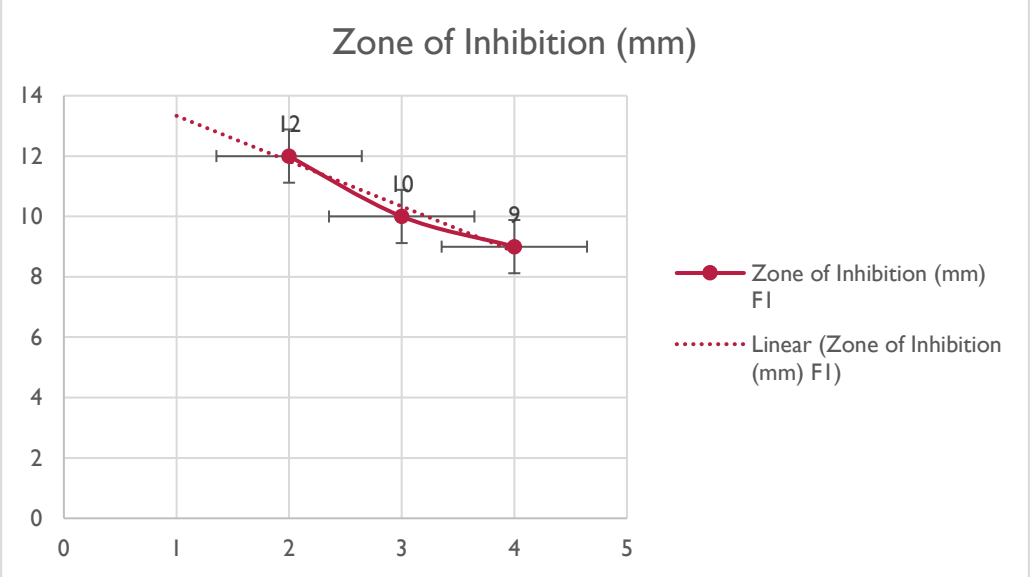


**Chart 4: Zone of inhibition of the prepared formulation by agar well**

- **Disc Diffusion Method:** This method reflected a similar trend, with F3 exhibiting the widest inhibition zones, followed by F2 and F1. The disc-impregnated formulations demonstrated consistent diffusion and effectiveness, verifying that the lotion serves as an efficient delivery system for the herbal extract.

**Table 9: Disc Diffusion Method**

| Sr. No | Organism | Zone of Inhibition (mm) |
|---|---|---|
| 1 | *Staphylococcus aureus* | 11 mm |
| 2 | *Escherichia coli* | 9 mm |
| 3 | *Pseudomonas aeruginosa* | 8 mm |

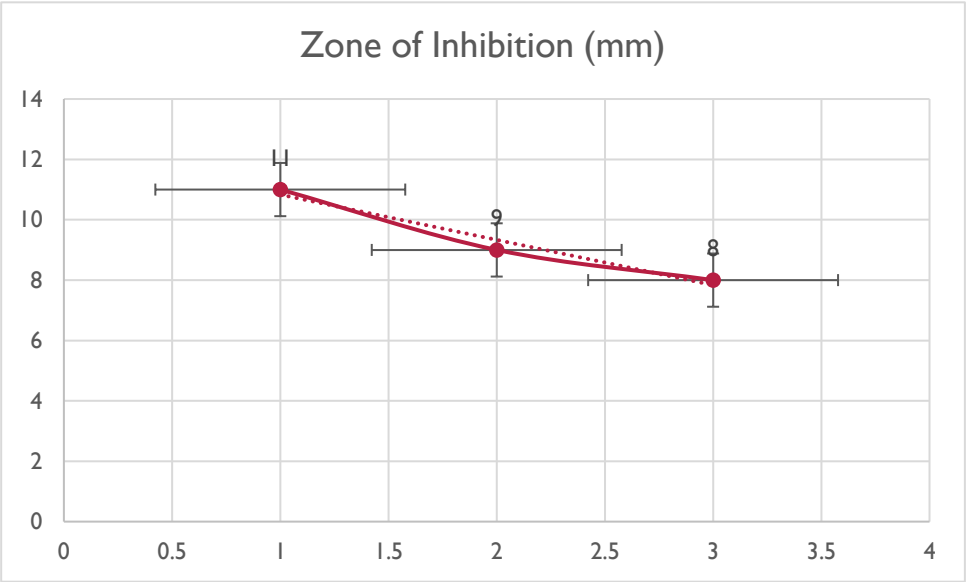


**Chart 5: Zone of inhibition of the prepared formulation by disc diffusion**

- **Broth Dilution Method (MIC):** The MIC test identified the minimum concentration at which visible bacterial growth was inhibited. The MIC values were:
  - 100 µg/mL for *Staphylococcus aureus*

- 125 µg/mL for *Escherichia coli*
- 150 µg/mL for *Pseudomonas aeruginosa*

These values reveal the concentration-dependent bacteriostatic effect of *Bauhinia racemosa*, validating its inclusion in topical formulations for antibacterial use.

**Table 10: MIC (Broth Dilution) Results**

| Sr. No. | Organism | MIC (Minimum Inhibitory Concentration) |
|---|---|---|
| 1 | *Staphylococcus aureus* | 100 µg/mL |
| 2 | *Escherichia coli* | 125 µg/mL |
| 3 | *Pseudomonas aeruginosa* | 150 µg/mL |

**Stability Study (90 Days) result:** Throughout the 90-day period, F3 remained physically stable with no phase separation, discoloration, or microbial contamination. The pH stayed within the acceptable skin-compatible range (5.5–6.2), and viscosity and spreadability remained consistent. Overall, the formulation showed excellent stability under all tested conditions, indicating its suitability for long-term storage and use.

**Table 11: Stability Study 90 Days results**

| Sr. No. | Parameter | Day 0 | Day 30 | Day 60 | Day 90 |
|---|---|---|---|---|---|
| 1 | Appearance | Smooth, off-white | No change | No change | No change |
| 2 | pH (RT) | 5.8 | 5.9 | 6.0 | 6.1 |
| 3 | pH (4°C) | 5.8 | 5.8 | 5.9 | 5.9 |
| 4 | pH (40°C) | 5.8 | 6.0 | 6.1 | 6.2 |
| 5 | Viscosity | Consistent | Consistent | Consistent | Consistent |
| 6 | Homogeneity | Uniform | Uniform | Uniform | Uniform |
| 7 | Microbial Growth | Absent | Absent | Absent | Absent |
| 8 | Phase Separation | No | No | No | No |

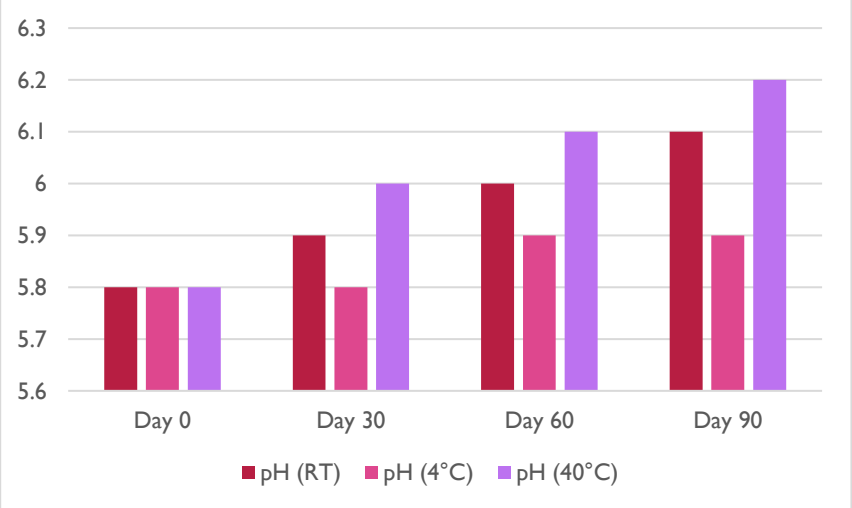


**Chart 6: Results of pH till 90 days**

**Best prepared formulation:** Among the three formulations evaluated, Formulation F3 (10% API) was found to be the most effective due to its superior antibacterial activity and optimal physicochemical properties. In both the agar well diffusion and disc diffusion methods, F3 exhibited the largest zones of inhibition against *Staphylococcus aureus*, *Escherichia coli*, and

*Pseudomonas aeruginosa*, confirming a concentration-dependent antibacterial effect. The MIC values further supported the efficacy of the extract, and F3 delivered a sufficient dose to inhibit bacterial growth effectively. Additionally, F3 maintained a skin-friendly pH, appropriate viscosity, excellent spreadability, uniform homogeneity, and remained stable under various storage conditions over a period of three months. These combined results demonstrate that F3 offers a potent, stable, and user-friendly topical formulation, making it the best choice for delivering the therapeutic benefits of *Bauhinia racemosa* in a lotion form.

**Conclusion:** The present study successfully demonstrated the extraction, phytochemical profiling, and formulation of a topical lotion using *Bauhinia racemosa* leaf extract. Qualitative screening and TLC confirmed the presence of bioactive compounds such as flavonoids, alkaloids, phenolics, and terpenoids. The lotion formulations (F1–F3) were stable, skin-compatible, and met standard evaluation parameters. Among them, F3 (10% API) showed superior antibacterial activity against *Staphylococcus aureus*, *E. coli*, and *Pseudomonas aeruginosa*. These findings support the therapeutic potential of *Bauhinia racemosa* in topical antibacterial formulations.

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