Volume 11, Issue 2, February – 2026 International Journal of Innovative Science and Research Technology
ISSN No: -2456-2165 https://doi.org/10.38124/ijisrt/26feb869

Formulation and Evaluation of Antimicrobial Activity of Suppositories by Using Cassia Fistula

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Publication Date: 2026/03/02

Abstract: This study evaluates herbal suppositories made from *Cassia fistula* (Golden Shower) extracts, which have inherent antibacterial characteristics. We created four formulations (F1, F2, F3, and F4) that combine gelatin, glycerin, and water with an ethanolic extract of plant flowers or leaves. The major goal was to evaluate the effectiveness of these suppositories against common infections by agar well diffusion and disc diffusion methods. The herbal suppositories demonstrated broad-spectrum antibacterial efficacy against *Staphylococcus aureus, Escherichia coli*, and *Candida albicans.*

How to Cite: Dr. M. Sakthivel; Dr. S. Mohamed Halith; A. Afrinbanu; S. Agalya; C. Ahalyamohan; S. Akash; S. Amina Banu (2026) Formulation and Evaluation of Antimicrobial Activity of Suppositories by Using Cassia Fistula. *International Journal of Innovative Science and Research Technology*, 11(2), 2153-2157. https://doi.org/10.38124/ijisrt/26feb869

I. INTRODUCTION

The rise of multidrug-resistant pathogens has created an urgent need for the development of novel antimicrobial agents. While synthetic antibiotics have long been the standard of care, their decreasing efficacy has led researchers to explore the vast therapeutic potential of medicinal plants . Traditional medicine systems, such as Ayurveda, have historically utilized botanical extracts to treat infections, providing a foundation for modern pharmacological investigation into natural bioactive compounds.

Among these plants, *Cassia fistula*, commonly known as the Golden Shower tree , stands out due to its diverse medicinal properties. This species is rich in secondary metabolites, including tannins, flavonoids, and anthraquinones, which are known to exhibit significant antibacterial and antifungal activity. While the antimicrobial properties of *Cassia fistula* extracts are well-documented, the challenge remains in delivering these benefits through effective and convenient dosage forms.

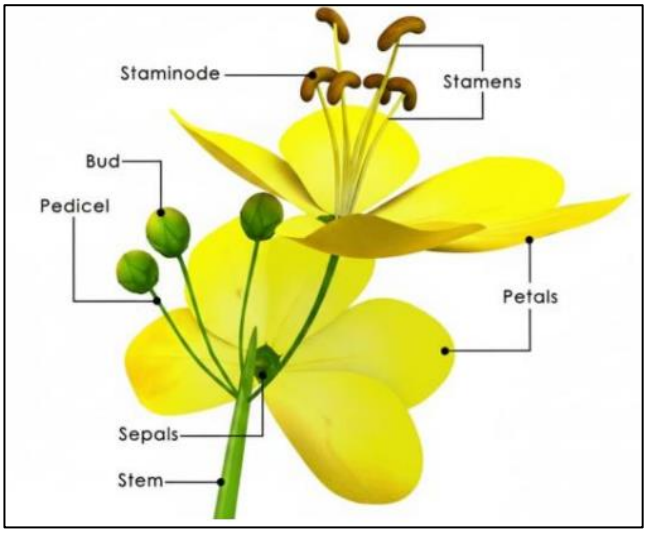

Fig 1 Morphological Features of *Cassia Fistula* (Golden Shower) Petals used for Ethanolic Extract

II. MATERIALS AND METHODS

- *Collection and Authentication:*

The floral specimens of *Cassia fistula L. (Fabaceae)* were collected for this study. The plant material was officially

ISSN No: -2456-2165 https://doi.org/10.38124/ijisrt/26feb869

identified and authenticated by Dr. S. Soosairaj, Associate Professor in the Department of Botany at St. Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu. The authentication was conducted on August 29, 2025, following the taxonomic guidelines outlined in the Flora of Northern and Central Tamil Nadu by John Britto S. (2019). A voucher specimen has been preserved for academic reference and can be accessed under the Reference Number 8173 at the Department of Botany, St. Joseph's College. Following authentication, the fresh flowers were cleaned and prepared for the ethanolic extraction process.

- *Preparation of Floral Extract:*

The collected flowers of *Cassia fistula* were thoroughly cleaned with distilled water to remove any surface impurities and subsequently shade-dried at room temperature. The dried floral material was then ground into a coarse powder using a mechanical grinder.

For the extraction process, a Soxhlet apparatus was utilized. Approximately 40 grams of the floral powder were placed in the siphon tube, and 95% ethanol was used as the menstrum. The extraction was carried out continuously for 24 hours to ensure the complete exhaustion of the bioactive constituents. Following the extraction, the resulting solution was collected in a beaker. To obtain the concentrated crude extract, the solvent was evaporated using a controlled heating method on a hot plate at a constant temperature of 30–40°C. This low-temperature evaporation was maintained until the solvent had completely dissipated, leaving behind a thick, concentrated ethanolic extract which was then stored in an airtight container at 4°C for further formulation.

Fig 2 Soxhlet Extraction

III. METHOD OF PREPARATION

- *Base Hydration:*

Weigh the required amount of gelatin and allow it to swell in purified water for a specific duration to ensure proper hydration.

- *Mixing the Base:*

Add the specified quantity of glycerin to the hydrated gelatin mixture.

- *Heating and Solubilization:*

Heat the mixture gently using a water bath, stirring constantly until a clear, homogenous liquid base is formed.

- *Incorporation of Extract:*

Allow the base to cool to a semi-solid but flowable state. Incorporate 500 mg of the ethanolic *Cassia fistula* floral extract and 100 mg of Tween 80, stirring continuously to ensure uniform dispersion of the active ingredients.

- *Molding:*

Pour the final medicated mixture into stainless steel suppository moulds that have been pre-lubricated to prevent sticking.

- *Solidification:*

Allow the moulds to cool at room temperature, then transfer them to a refrigerator at 4°C for complete solidification.

- *Demolding and Storage:*

Carefully remove the solidified suppositories from the moulds and store them in an airtight container for subsequent evaluation.

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Table 1 Formulation of Suppositories

S.NO	INGREDIENTS	FORMULATION CODE			
		F1	F2	F3	F4
1	Cassia fistula extract (mg)	500	500	500	500
2	Gelatin(g)	1.5	1.4	1.4	1.3
3	Glycerin(mg)	100	200	500	100
4	Tween 80(mg)	100	100	100	100
5	Distilled water(ml)	q.s.	q.s.	q.s.	q.s.

➢ *Evaluation of Suppositories*

- *Determination of Anti-Microbial Activity:*

Preparation of bacterial and fungal strains: *Staphylococcus aureus* (MTCC 3160), *Escherichia coli* (MTCC 732), *Candida albicans* (MTCC 183) from a collection of microbial cultures at IMI (Chandigarh, India). All bacterial strains were cultured in Muller Hinton broth (MHB) at 37 degrees Celsius for 24 hours with 200-degree agitation at a rate of 50 rpm. All fungal strains were cultured in potato dextrose broth at 37 degrees Celsius for 24 hours.

- *Agar Well Diffusion Method:*

Agar Well Diffusion Susceptibility Test Method (ABDT) as followed by NCCLS (1993) and *Awoyinka et al.*, (2007). Microbial strains were seeded on Mueller-Hinton agar (MHA) and PDA (Merck, Germany) using sterile cotton swabs. Using sterile forceps, filter papers (with a diameter of 6 mm) containing 25, 50 or 100 micrograms of AgNPs and standard solutions such as Streptomycin and Fluconazole.

IV. RESULTS AND DISCUSSION

The results and discussion of the screening of *Cassia fistula's* antimicrobial activity show that the ethanolic extract of *Cassia fistula* flowers and the prepared herbal suppositories of different concentrations have antimicrobial activity against a variety of microorganisms, including fungi like *Candida albicans* (MTCC 183), Gram-positive bacteria like *Staphylococcus* aureus (MTCC 3160), and Gram-negative bacteria like *Escherichia coli* (MTCC 732).

➢ *Antibacterial Evaluation*

Evaluation of Antibacterial Activity of *Cassia fistula* Suppositories

Activity against Gram-Positive Bacteria (Staphylococcus *aureus)*

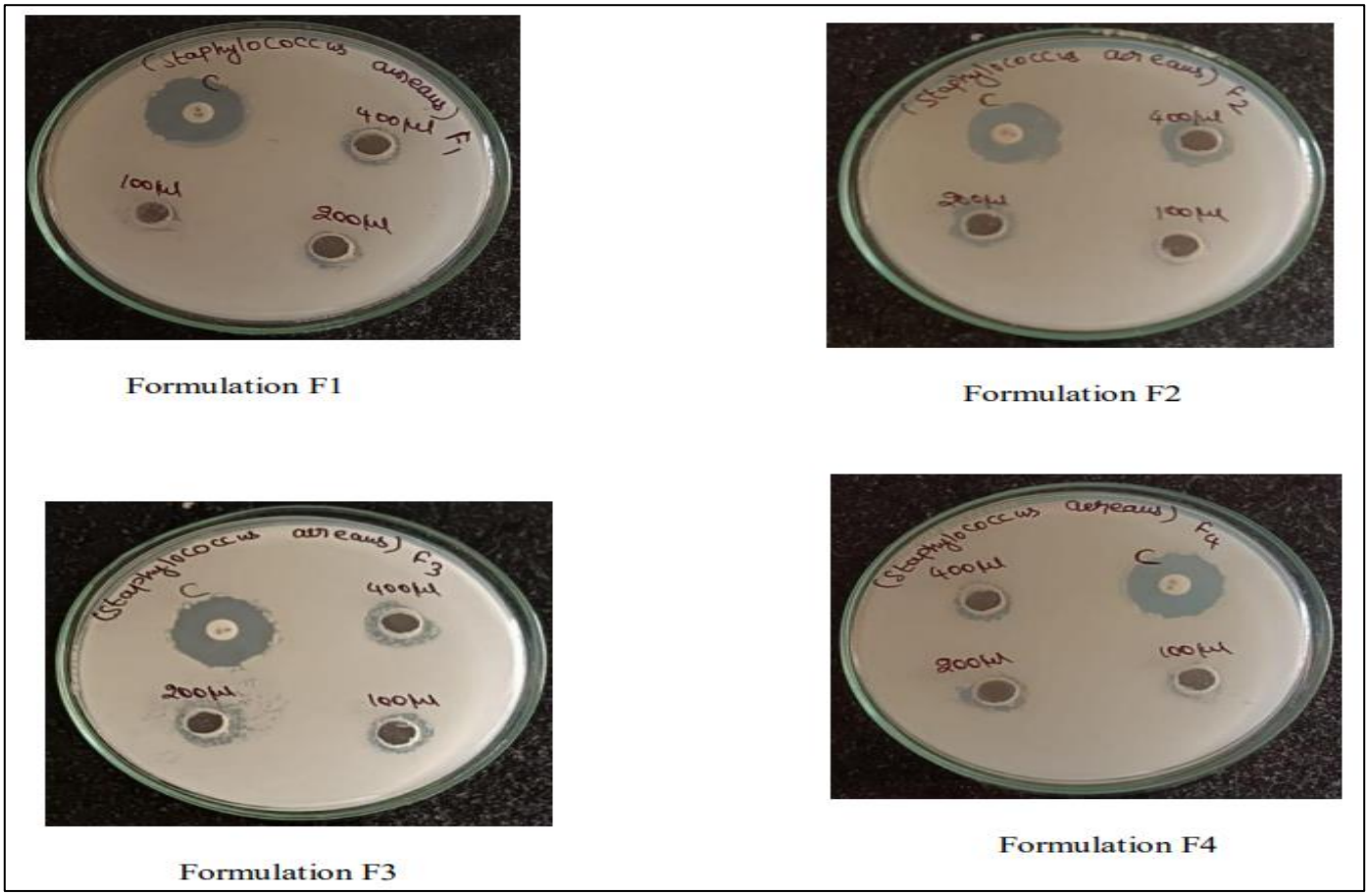

Fig 3 Comparison of the Zones of Inhibition for Formulations F1 Through F4 Against Gram-Positive *Staphylococcus Aureus.*

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Activity against Gram-Negative Bacteria

(Escherichia coli)

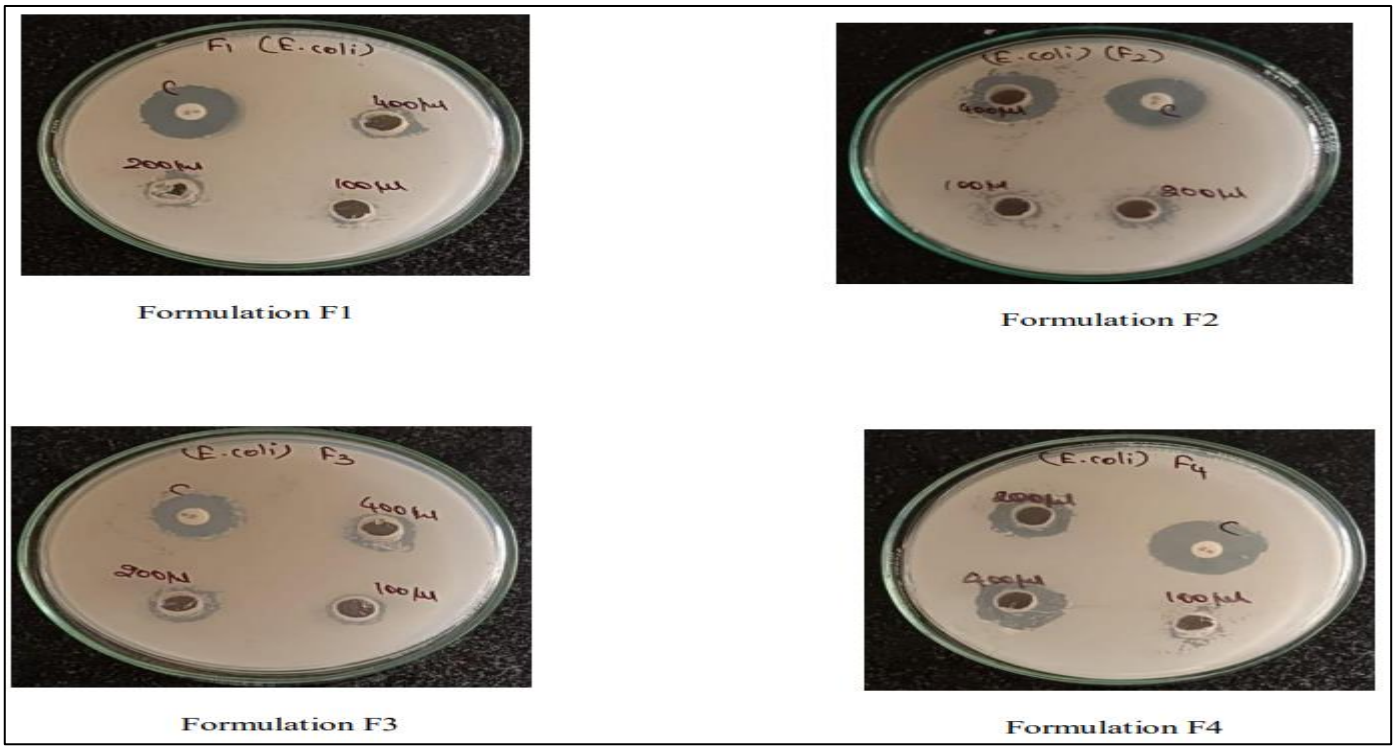

Fig 4 Antibacterial Activity of Herbal Suppositories Against *Escherichia Coli."*

- *Antifungal Evaluation*

  Evaluation of Antifungal Activity of *Cassia fistula* Suppositories

Activity Against *Candida albicans*

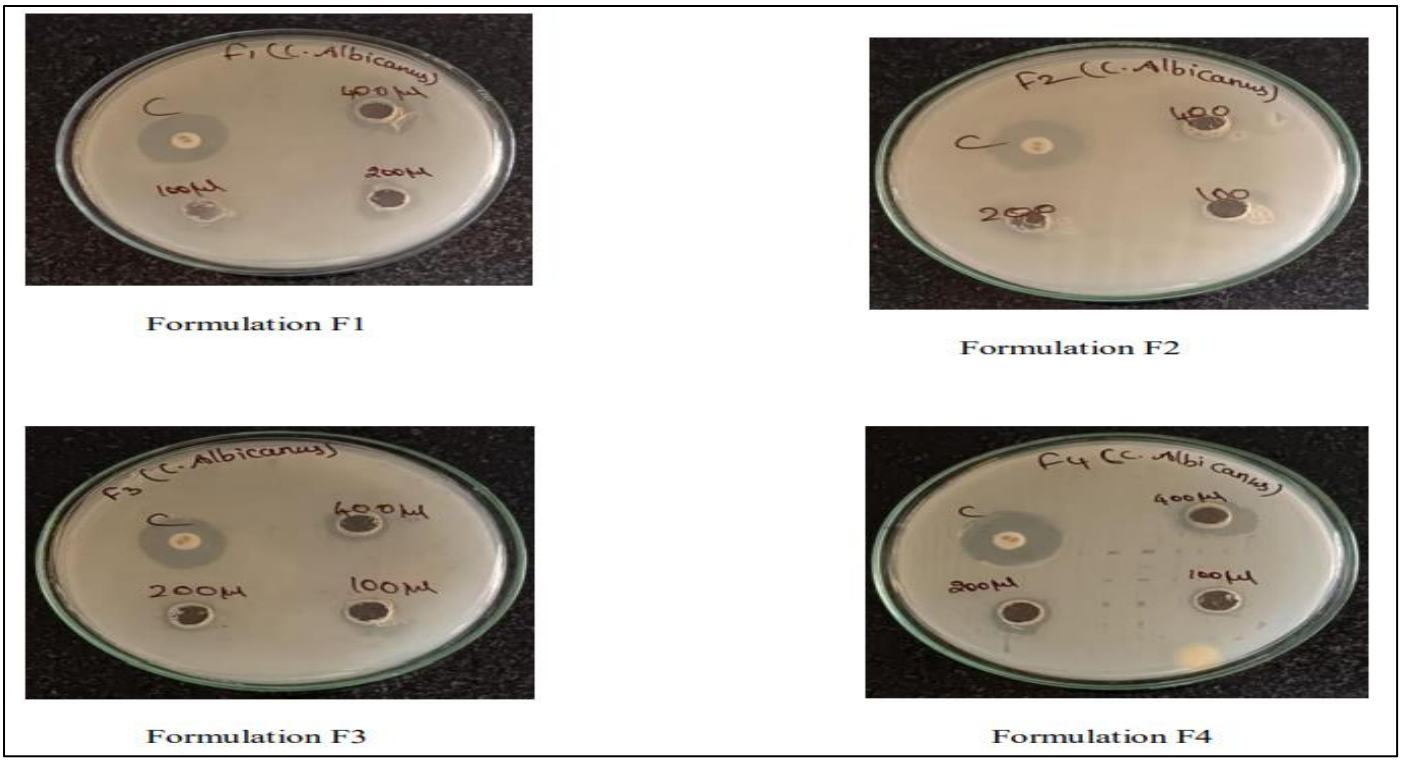

Fig 5 Antifungal Efficacy of the Formulated Suppositories Against *Candida Albicans."*

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V. CONCLUSION

The study's findings indicate that by employing an ethanolic extraction process with a Soxhlet apparatus, *Cassia fistula* can be efficiently prepared into a semisolid suppository dosage form with strong therapeutic activity. The plant *"Cassia fistula"* was found to contain the active antimicrobial macromolecules. Therefore, it was determined that the herbal suppositories showed distinct zones of inhibition against fungal *(Candida albicans)*, Gram-negative *(Escherichia coli)*, and Gram-positive *(Staphylococcus aureus)* pathogens.

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