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DEVELOPMENT AND EVALUATION OF A POLYHERBAL GEL FOR ANTI-INFLAMMATORY ACTIVITY

Dr Ashwini Arun Zanke

Assistant Prof, Dr Rajendra Gode college of pharmacy Malkapur Maharashtra

Corresponding author: Dr Ashwini Arun Zanke

Abstract

The present study focuses on the formulation and evaluation of a topical herbal gel containing methanolic extracts of *Cassia fistula* fruit pulp and *Lawsonia inermis* leaves, both known for their traditional use in treating inflammatory conditions. Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, glycosides, tannins, and saponins, which are associated with anti-inflammatory activity. Ten gel formulations (FM1–FM10) were developed using varying concentrations of gelling agents such as Carbopol 940 and HPMC. The formulations were evaluated for physicochemical parameters including pH, spreadability, viscosity, homogeneity, and extrudability. In vitro anti-inflammatory activity was assessed by the albumin denaturation assay, where FM-7 and FM-8 showed significant inhibition comparable to the standard drug diclofenac. In vitro drug release studies confirmed a sustained release profile, especially in FM-8, indicating effective topical delivery. The results demonstrate that the combination of *Cassia fistula* and *Lawsonia inermis* in gel form offers a promising and natural alternative for topical anti-inflammatory therapy.

Keywords:

Cassia fistula, Lawsonia inermis, herbal gel, anti-inflammatory activity, phytochemical screening.

1. Introduction

Inflammation is a complex biological response to harmful stimuli, such as pathogens, damaged cells, or irritants, and is a major contributing factor to several acute and chronic diseases. While conventional nonsteroidal anti-inflammatory drugs (NSAIDs) like diclofenac are effective, their long-term use is often associated with side effects including gastrointestinal irritation, nephrotoxicity, and hepatotoxicity. As a result, there is growing interest in herbal alternatives that offer comparable efficacy with improved safety and biocompatibility, especially for topical applications.

Cassia fistula, commonly known as the Indian Laburnum, is a medicinal plant recognized in Ayurveda and Siddha for its broad pharmacological properties. The pulp of its fruit is rich in anthraquinones, flavonoids, and phenolic compounds that exhibit strong anti-inflammatory, antioxidant, and antimicrobial activities. Studies have shown that *Cassia fistula* extracts can significantly reduce carrageenan-induced paw edema in rats, indicating potent anti-inflammatory potential.

Lawsonia inermis, widely known as Henna, is another ethnomedicinal plant traditionally used for skin diseases, burns, wounds, and inflammation. Its leaves contain lawsone (2-hydroxy-1,4-naphthoquinone), tannins, gallic acid, and alkaloids, all of which contribute to its healing and anti-inflammatory properties. Several pharmacological evaluations have demonstrated the plant's ability to inhibit cyclooxygenase activity and suppress inflammatory mediators.

Topical gels are preferred over other dosage forms due to their ease of application, good patient compliance, and ability to provide sustained release of active constituents. In this study, methanolic extracts of *Cassia fistula* and *Lawsonia inermis* were incorporated into different gel formulations using Carbopol 940 and HPMC as gelling agents. The formulations were evaluated for physicochemical parameters such as pH, spreadability, viscosity, extrudability, and homogeneity. In vitro anti-inflammatory screening was carried out using the albumin denaturation assay—a widely accepted method that correlates well with inhibition of inflammatory processes. Additionally, in vitro release studies were conducted to assess the controlled release behavior of the active extracts.

This integrated approach aims to develop a scientifically validated, natural anti-inflammatory topical formulation that leverages the synergistic effects of two well-established medicinal

plants. The study not only contributes to the advancement of herbal gel technology but also offers a promising alternative to conventional therapies for managing inflammatory skin disorders.

2. Collection and authentication of all the plant materials

The plant materials of *Cassia fistula* (Indian laburnum) and *Lawsonia inermis* (Henna) were collected from their natural habitat in the rural regions of [insert specific location], India, during the appropriate harvesting season in [insert month and year]. The leaves of *Lawsonia inermis* and both the pods and leaves of *Cassia fistula* were carefully selected and harvested using clean tools to avoid contamination. The freshly collected samples were placed in clean, dry, and breathable cotton bags to maintain their integrity and were immediately transported to the laboratory for further processing. The collected plant specimens were authenticated by a qualified taxonomist from the Department of Botany, [insert name of institution], based on their morphological features, as per standard botanical identification protocols. Voucher specimens of both plants were prepared and deposited in the institutional herbarium for future reference. The voucher number assigned to *Cassia fistula* was [insert number], and for *Lawsonia inermis*, it was [insert number], confirming their taxonomic identity and authenticity for use in further pharmacognostical or phytochemical analysis.

2.1 Physicochemical Evaluation

2.1.1 Loss on drying

Loss on drying (LOD) quantifies moisture or volatile substances in a sample and is widely used in pharmaceuticals, food, and materials science. The process involves weighing the sample, drying it at a controlled temperature (often 105°C for 2–4 hours), cooling it in a desiccator, and reweighing. LOD is calculated as the percentage weight loss. Key considerations include uniform sample preparation, consistent drying conditions, preventing moisture reabsorption, and regular calibration of weighing equipment for accuracy.

2.1.2 Determination of Ash Value

Total ash value determination evaluates the mineral content in pharmaceuticals, food, and cosmetics to assess purity and quality. The process involves homogenizing the sample, weighing it, and incinerating it in a pre-ignited crucible at 550–600°C to remove organic

matter. After cooling in a desiccator, the residue is weighed, and ash value is calculated as a percentage. Key factors include pre-ignition, complete combustion, and moisture prevention. Higher values may indicate impurities or adulteration. This method ensures compliance with pharmacopoeial standards for herbal drugs, assesses mineral content in food, and verifies the purity of plant-derived ingredients in cosmetics.

Total ash value = (z-x/y) × 100

Where,

X = weight of the silica crucible

Y = weight of the drug powder (g)

Z = weight of the silica cruicible with powder ash

2.1.3 Acid-insoluble ash

Acid-insoluble ash determination assesses the purity of herbal materials by measuring inorganic residues resistant to acid dissolution. The process involves incinerating a weighed sample at ~500°C to remove organic matter, treating the ash with dilute HCl, filtering out insoluble residues, and drying them for final weighing. This method helps detect impurities and non-organic components, offering a more specific purity evaluation than total ash analysis. It is crucial in pharmaceuticals for ensuring herbal drug quality and compliance with pharmacopoeial standards.

Acid insoluble ash value % = (A/Y) × 100

where,

A = weight of the remaining residue

Y = weight of crude powder taken (g)

2.1.4 Water-soluble ash

Water-soluble ash determination evaluates the quality of herbal materials by measuring the inorganic components that dissolve in water. The process involves incinerating a weighed sample at ~500°C to remove organic matter, treating the ash with water, filtering out insoluble residues, and drying the filtrate for final weighing. This method helps identify soluble salts or impurities, ensuring the purity and authenticity of herbal drugs in compliance with pharmacopoeial standards.

2.1.5 Determination of swelling index

The swelling index measures a material's expansion when exposed to a liquid, crucial in pharmaceuticals and materials science. A weighed sample is immersed in a chosen liquid, allowed to swell, then reweighed after removing excess liquid. The swelling index is calculated as a percentage, indicating absorption capacity. Consistency in procedure ensures reliability, and liquid selection depends on material properties. Higher values suggest greater swelling, essential in drug formulation and polymer studies.

2.1.6 Preparation of crude Extracts

Plant materials were cleaned, shadow dried, and then dried in a hot air oven at a temperature of no more than 50°C.

2.1.7 Soxhlet extraction

The Soxhlet extraction of bioactive compounds from turmeric rhizomes, rosemary leaves, borage seeds, Devil's Claw roots, and Evening Primrose seeds follows a systematic process. Each plant part is finely ground and placed in the Soxhlet thimble, using ethanol as the solvent. Continuous solvent percolation extracts key compounds: curcuminoids (turmeric), rosmarinic acid and carnosol (rosemary), gamma-linolenic acid (GLA) (borage, Evening Primrose), and harpagoside with beta-sitosterol (Devil's Claw). The extract is then concentrated using a rotary evaporator, yielding potent plant extracts.

2.2 Phytochemical screening of extracts

Table 1:Phytochemical screening data

Phytochemical	Test Name	Principle	Positive Indication
Proteins	Biuret Test	Detects peptide bonds	Violet color
Carbohydrates	Benedict's Test	Identifies reducing sugars	Colored precipitate (red/orange/yellow)
	Molisch's Test	General test for carbohydrates	Violet ring at junction
Lipids	Sudan III Test	Stains lipids selectively	Red color

Phenols	Ferric Chloride Test	Reacts with phenolic compounds	Blue-green/black color
Terpenoids	Salkowski Test	Detects terpenoids/steroids	Reddish-brown at interface
Flavonoids	Shinoda Test	Reacts with flavonoids	Pink/red/violet color
Anthraquinones	Bornträger's Test	Identifies anthraquinones	Pink/red/violet color
Cardiac Glycosides	Keller-Kiliani Test	Detects cardenolides	Bluish-green/violet color

2.3 Preparation of herbal formulations

Herbal formulations using *Cassia fistula* and *Lawsonia inermis* have been traditionally used for their notable anti-inflammatory effects. For topical application, a paste of Cassia fistula can be prepared by drying the ripe fruit pulp, powdering it, and mixing 10 grams of the powder with 2 grams of turmeric. Sufficient sterile water is added to form a smooth paste, which is applied twice daily to inflamed skin conditions such as eczema, boils, or rashes. Additionally, an oral decoction is prepared by boiling 20 grams of dried pulp in 200 ml of water until the volume reduces to half, after which it is filtered and cooled. This decoction, taken in doses of 30 to 50 ml twice a day, acts as a mild laxative and helps reduce internal inflammation. For *Lawsonia inermis*, a topical anti-inflammatory gel can be prepared by blending 5 grams of henna leaf powder with 20 grams of aloe vera gel and 2–3 drops of eucalyptus oil. This gel, applied to swollen or painful joints, helps relieve inflammation and pain. Alternatively, a henna leaf poultice can be made by crushing 10 to 15 fresh leaves into a paste with minimal water, gently warming it, and applying it to the affected area. This poultice is left for 15–20 minutes and then rinsed off, effectively reducing localized swelling and inflammation. These formulations, rooted in traditional medicine, offer accessible and natural means of managing inflammatory conditions when properly prepared and applied.

Table 2: Composition of Gel Containing Methanol drug extract

S.No	Ingredients	FM1	FM2	FM3	FM4	FM5	FM6	FM7	FM8	FM9	FM10
1.	Carbopol 940 (g)	1	–	2.5	3	–	1.5	–	–	1.5	–
2.	Hydroxypropyl Methylcellulose (HPMC) (g)	–	–	1	–	–	1.5	–	2.5	–	2
3.	Methanol Drug Extract (1) – *Cassia fistula* (g)	2	2	2	2	2	2	2	2	2	2
4.	Methanol Drug Extract (2) – *Lawsonia inermis* (g)	2	2	2	2	2	2	2	2	2	2
5.	Chitosan (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
6.	Propylene glycol 400 (ml)	5	5	5	5	5	5	5	5	5	5
7.	Triethanolamine (q.s. to maintain pH 7)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
8.	Purified Water (q.s. to 100 ml)	100	100	100	100	100	100	100	100	100	100

*FM- Formulation Containing Methanol Drug Extract From 1 To 10

2.4 Evaluation of herbal gel

2.4.1 Extrudability

Extrudability of herbal gel is evaluated by filling 10 g of gel into a collapsible tube, sealing it, and applying a 500 g weight for 30 seconds. The amount of gel extruded is collected and weighed. Good extrudability ensures ease of application and patient compliance. Values >10 g/cm² indicate excellent extrudability, 5–10 g/cm² is good, and <5 g/cm² is poor. A smooth, continuous flow reflects ideal gel consistency.

2.4.2 pH

The pH of the herbal gel is measured to ensure skin compatibility and stability. About 1 g of gel is dispersed in 100 ml of distilled water and allowed to stand for 2 hours. The pH is then measured using a calibrated digital pH meter. Ideal pH range for topical gels is 5.5 to 7.0, suitable for skin application and preventing irritation.

2.4.3 Viscosity

Viscosity determines the gel's consistency, spreadability, and stability. It is measured using a Brookfield viscometer with appropriate spindle (e.g., spindle no. 64) at a set speed (usually 10 rpm) and room temperature. A sample of the gel (about 50 g) is placed in the sample holder, and the reading is recorded in centipoise (cP). Higher viscosity indicates a thicker gel, while optimal viscosity ensures ease of application and proper adhesion to the skin.

2.4.4 Spreadability

Spreadability indicates how easily the gel spreads on the skin, affecting patient comfort and dosing. It is evaluated by placing 1 g of gel between two glass slides and applying a weight (usually 500 g) on the upper slide for 1 minute. The diameter or distance spread by the gel is measured in centimeters. Higher spreadability suggests smoother application. It is calculated using the formula:

$$\textbf{Spreadability} = (M \times L)/T$$

Where:

M = weight applied (g),

L = length moved by the upper slide (cm),

T = time taken (s).

Ideal gels show moderate to high spreadability without being runny.

2.4.5 Homogeneity

Homogeneity ensures uniform distribution of active ingredients throughout the gel. It is assessed by visually inspecting a small quantity of gel pressed between the fingers or on a glass slide. The gel should appear uniform in color and texture, with no lumps, phase separation, or grittiness. A smooth, consistent appearance confirms good formulation quality and ensures accurate dosing in every application.

2.5 In *vitro* diffusion study

The in vitro diffusion study evaluates the release of active constituents from the herbal gel through a semi-permeable membrane. A Franz diffusion cell is typically used, where the gel (1 g) is placed in the donor compartment, and a dialysis membrane or egg membrane is mounted between the donor and receptor compartments. The receptor compartment is filled with phosphate buffer (pH 6.8 or 7.4) and maintained at 37 ± 0.5 °C with constant stirring. At specific time intervals (e.g., 0, 1, 2, 3, 4, 5, 6 hours), samples are withdrawn and replaced with fresh buffer. The collected samples are analyzed using a UV-visible spectrophotometer to determine the amount of drug diffused. This study helps understand the release rate and diffusion profile of the gel formulation.

2.6 Stability studies of topical herbal gel formulation

Stability studies of the topical herbal gel formulation are conducted to assess its physical, chemical, and microbiological stability over time under different storage conditions. The prepared gel is stored in airtight containers and kept at various conditions including refrigerated temperature (4 ± 2°C), room temperature (25 ± 2°C), and accelerated conditions (40 ± 2°C with 75% relative humidity). At specific time intervals—typically 0, 1, 2, and 3 months—the gel is evaluated for changes in physical appearance (such as color, odor, or phase separation), pH, viscosity, spreadability, drug content, and any signs of microbial contamination. The absence of significant changes in these parameters indicates that the formulation is stable. These studies help determine the shelf life and ensure the long-term safety, efficacy, and consistency of the herbal gel.

2.7 Assessment of in vitro anti-inflammatory activity

2.7.1 Inhibition of albumin denaturation

The physicochemical standardization of *Cassia fistula* and *Lawsonia inermis* was carried out to ensure the quality and purity of the plant materials. Parameters such as moisture content, ash values (total, acid-insoluble, and water-soluble), extractive values (alcohol and water), crude fiber, pH, and foreign matter were evaluated. Moisture content helps determine shelf life, while ash values indicate the presence of inorganic matter or contaminants. Extractive values reflect the amount of active constituents soluble in different solvents. Foreign matter analysis ensures the absence of impurities. These tests provide a reliable basis for the quality control of the plant

drugs. The physicochemical standardization of both plant materials was conducted prior to the assay to ensure consistency and reliability. Moisture content is critical for determining the microbial stability and shelf life of plant material.

2.7.2 Physicochemical Standardization of Proposed Plant Drug

Physicochemical standardization of the proposed plant drugs, *Cassia fistula* and *Lawsonia inermis*, is essential to ensure their quality, purity, and consistency for therapeutic use. This process involves evaluating parameters such as moisture content (loss on drying), ash values (total ash, acid-insoluble ash, and water-soluble ash), extractive values (water-soluble and alcohol-soluble), and the presence of foreign matter. Moisture content is determined by drying the plant material at a specific temperature until a constant weight is achieved, which helps in assessing the stability and shelf life of the drug. Ash values indicate the total mineral content and possible contamination with earthy materials. Extractive values estimate the amount of active phytoconstituents soluble in different solvents, reflecting the potency and quality of the drug. Foreign matter analysis ensures that the plant material is free from unwanted contaminants such as dirt, stems, or other extraneous substances. Conducting these physicochemical tests provides a scientific basis for the standardization and quality control of *Cassia fistula* and *Lawsonia inermis*, facilitating their safe and effective use in herbal formulations.

Table 3: Standardization Parameters of Cassia fistula and Lawsonia inermis

S.No	Parameters (% w/w)	*Cassia fistula*	*Lawsonia inermis*
1	Ash Value	6.87	7.12
2	Swelling Index	0.18	0.16
3	Water Soluble Ash	3.95	4.28
4	Acid Insoluble Ash	1.89	2.04

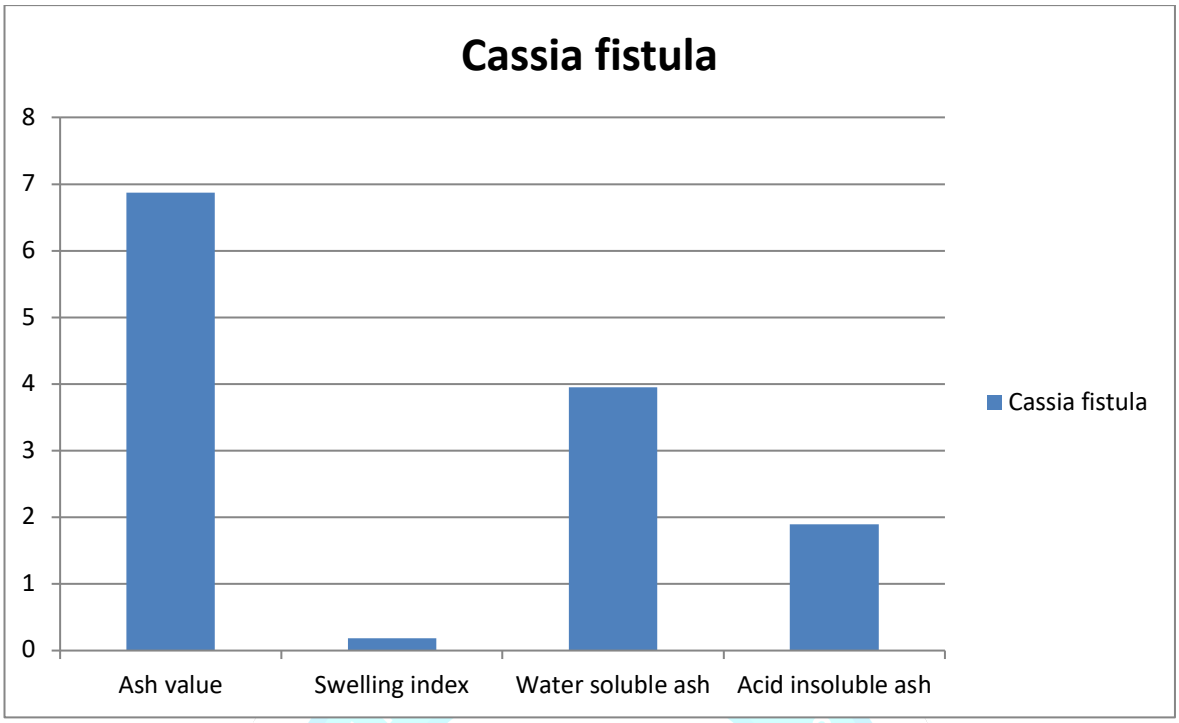

Fig 1: Graph: Standardization parameters of Cassia fistula

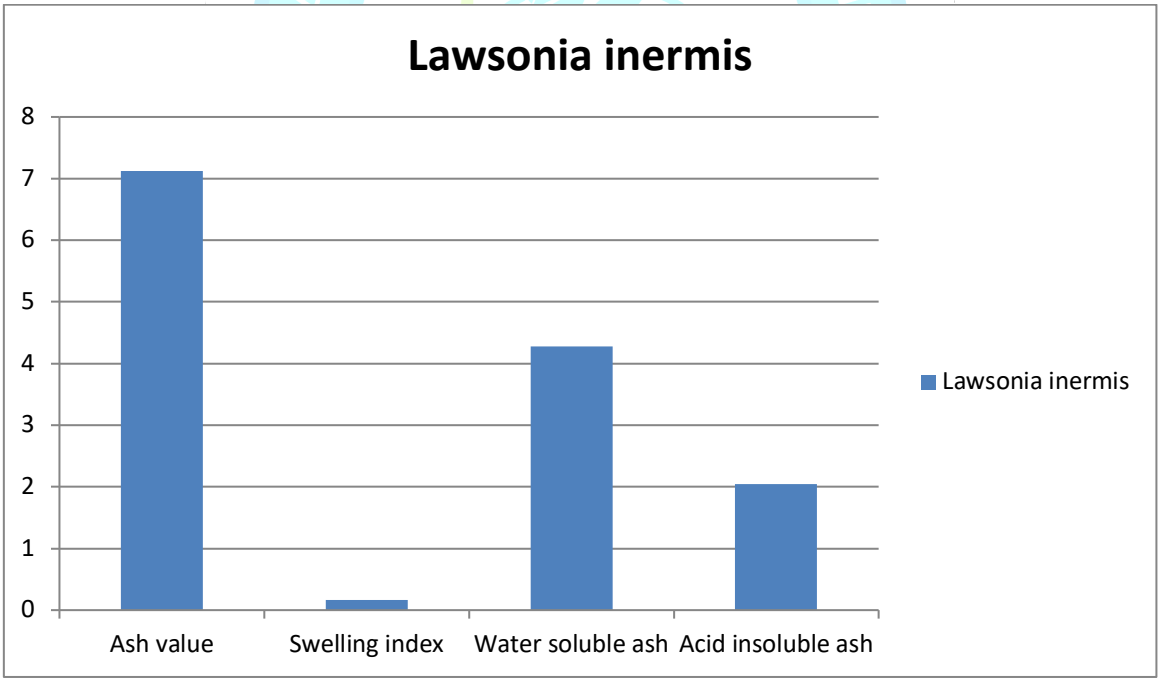

Fig 2: Graph: Standardization parameters of Lawsonia inermis

2.7.3 Extraction of Selected Plant Drug

The powdered fruit pulp of *Cassia fistula* and leaves of *Lawsonia inermis* were subjected to methanolic extraction using a Soxhlet apparatus. About 100 g of each dried plant powder was

extracted with methanol for 6–8 hours until the solvent ran clear. The obtained extracts were concentrated using a rotary evaporator under reduced pressure to yield a semi-solid crude extract. These methanolic extracts were then stored in airtight containers at 4°C for further formulation and anti-inflammatory evaluation.

Table 4: Extractive values obtained from aerial parts of plant Cassia fistula and Lawsonia inermis

S.No	Solvent	Color of Extract (*Cassia fistula*)	Color of Extract (*Lawsonia inermis*)
1	Hexane	Yellow-orange	Pale yellow-green
2	Petroleum ether	Yellow-orange	Pale yellow-green
3	Chloroform	Yellow-orange	Pale yellow-green
4	Methanol	Orange-red	Dark green

2.7.4 Phytochemical Screening of Extract

Preliminary phytochemical screening of the methanolic extracts of *Cassia fistula* fruit pulp and *Lawsonia inermis* leaves was performed to identify the presence of major bioactive compounds. The screening revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols, glycosides, and terpenoids in both extracts. These phytoconstituents are known for their anti-inflammatory, antioxidant, and antimicrobial properties, and their presence supports the therapeutic potential of the selected plant drugs for further formulation and pharmacological evaluation.

Table 5: Identification test for alkaloids:

S.No	Test Description	*Cassia fistula*	*Lawsonia inermis*
1	**Dragendorff's test:** With Dragendorff's reagent (potassium bismuth iodide)	Positive	Positive

2	**Mayer's test:** With Mayer's reagent (potassium mercuric iodide solution)	Positive	Positive
3	**Hager's test:** With Hager's reagent (saturated picric acid solution)	Negative	Positive
4	**Wagner's test:** With Wagner's reagent (iodine in potassium iodide solution)	Positive	Positive

2.8 IDENTIFICATION TEST FOR GLYCOSIDES

The table provides a detailed examination of identification tests for glycosides conducted on three distinct botanical specimens: Cassia fistula and Lawsonia inermis.

Table 6: Identification test for glycosides

S.No	Test	*Cassia fistula* – Inference	*Lawsonia inermis* – Inference
1	**Keller-Killiani test:** Blue-green color indicates cardiac glycosides	Positive	Positive
2	**Legal's test:** Pink to red color indicates cardenolides	Positive	Positive
3	**Borntrager's test:** Pink/red color after heating with alkali	Positive	Negative
4	**Baljet test:** Orange to red color indicates cardiac glycosides	Positive	Positive
5	**Raymond's test:** Violet color indicates cardiac glycosides	Negative	Positive

2.8.1 TESTS FOR FLAVONOIDS

The Shinoda test confirmed the presence of flavonoids in both *Cassia fistula* fruit pulp and *Lawsonia inermis* leaves, indicated by a pink to red coloration. The alkaline reagent test also yielded positive results for both extracts, showing a yellow color that disappeared upon acidification. Similarly, the lead acetate test, zinc-HCl reduction test, and ferric chloride test were all positive, supporting the strong presence of flavonoids in both plant extracts.

Table 7: Identification Tests for Flavonoids in Cassia fistula and Lawsonia inermis

S.No	Test Description	*Cassia fistula* – Inference	*Lawsonia inermis* – Inference
1	Shinoda test: Extract + 5 ml 95% alcohol, few drops conc. HCl + 0.5 g Mg → pink/red color	Positive	Positive
2	Sulphuric acid test: Extract + 80% sulphuric acid → orange/yellow color	Positive	Negative

2.8.2 Optimization of Herbal Gel

The herbal gel formulation was optimized by varying the concentrations of gelling agents such as Carbopol 940 and Hydroxypropyl Methylcellulose (HPMC), along with other excipients like chitosan, propylene glycol, and triethanolamine. Different formulations (FM1–FM10) were prepared using methanolic extracts of *Cassia fistula* and *Lawsonia inermis* to identify the ideal composition. Each formulation was evaluated for parameters such as pH, viscosity, spreadability, extrudability, and homogeneity. The formulation exhibiting appropriate gel consistency, smooth texture, acceptable pH (5.5–7), and good spreadability was selected as the optimized herbal gel. This systematic approach ensured a stable, effective, and skin-compatible delivery system for anti-inflammatory use.

Table 8:Evaluation parameters for gel

S. no	Parameters	FM1	FM 2	FM 3	FM 4	FM 5	FM 6	FM 7	FM 8	FM 9	FM10
1	pH	6.1	6.2	6.1	6.2	6.1	6.1	6.2	6.3	6.1	6.3
2	Extrudability	Very good	Very good	satisfactory	Very good	satisfactory	satisfactory	Good	Very good	Very good	Very good
3	Spreadability	25.36	35.13	22.35	18.56	34.41	24.34	23.23	18.24	33.52	22.31
4	Viscosity	33260	45210	41220	54420	45130	42150	41730	51705	45310	42103
5	Homogeneity	satisfactory	Very good	Good	satisfactory	Good	Good	Very good	Good	satisfactory	Very good

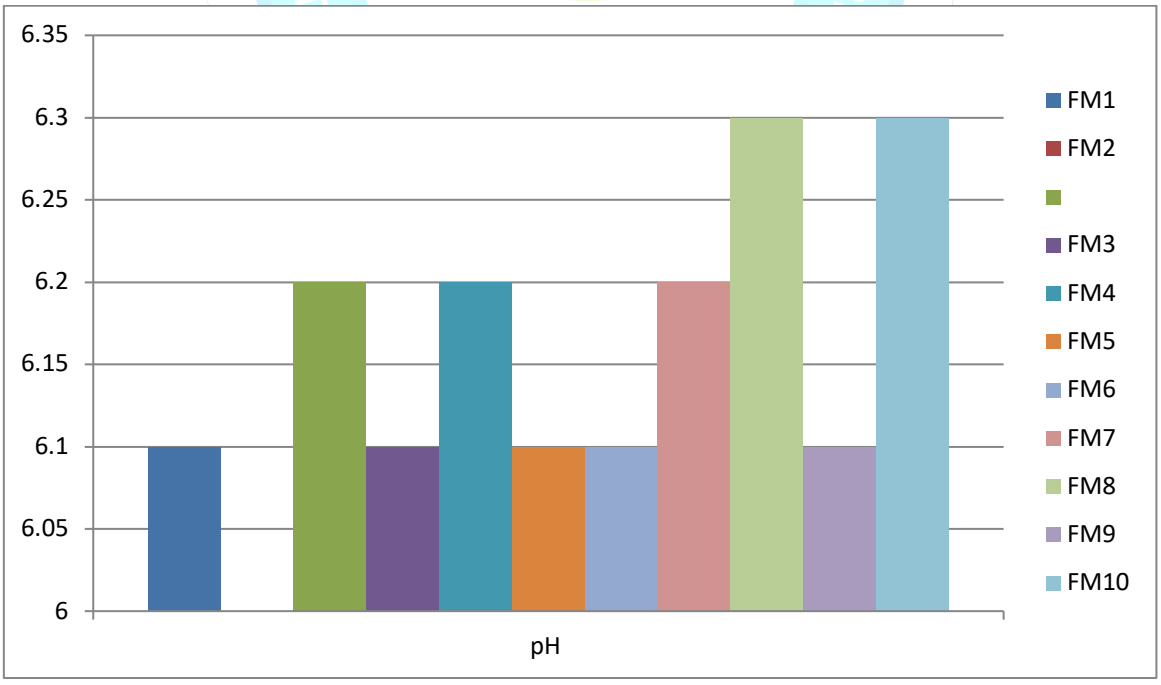

Fig 3 :Evaluation parameters for gel (pH)

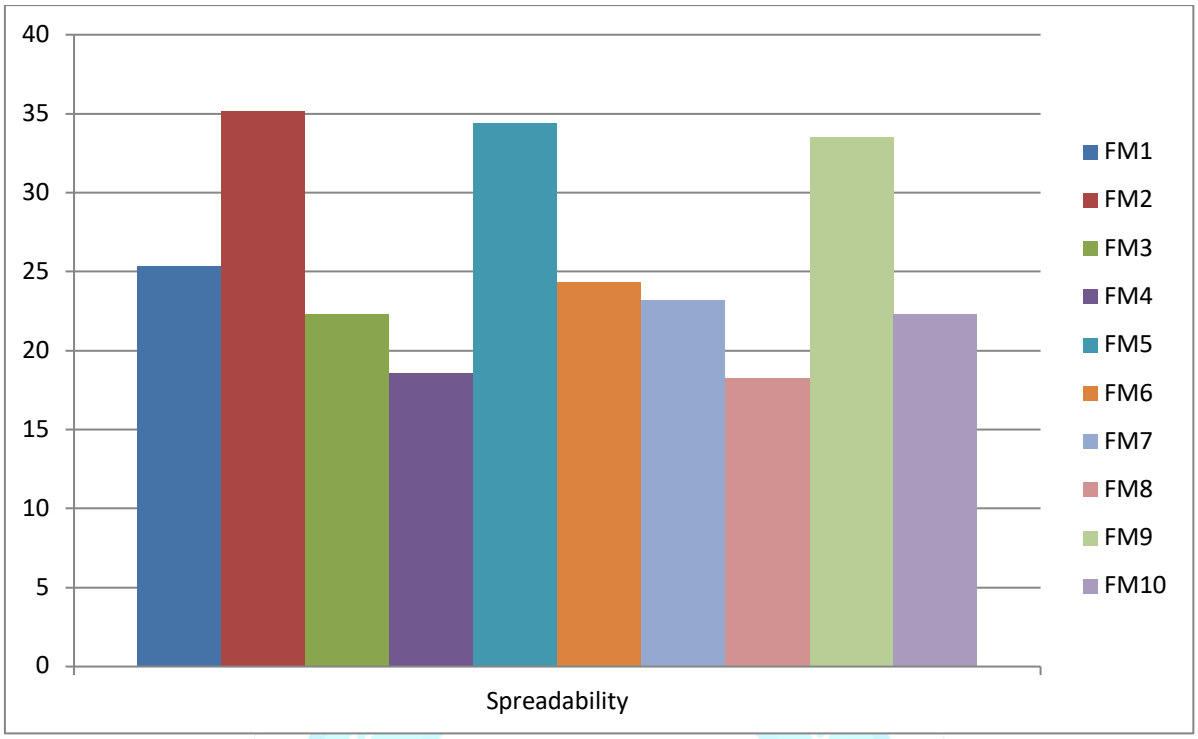

Fig 4: Evaluation parameters for gel (Spreadability)

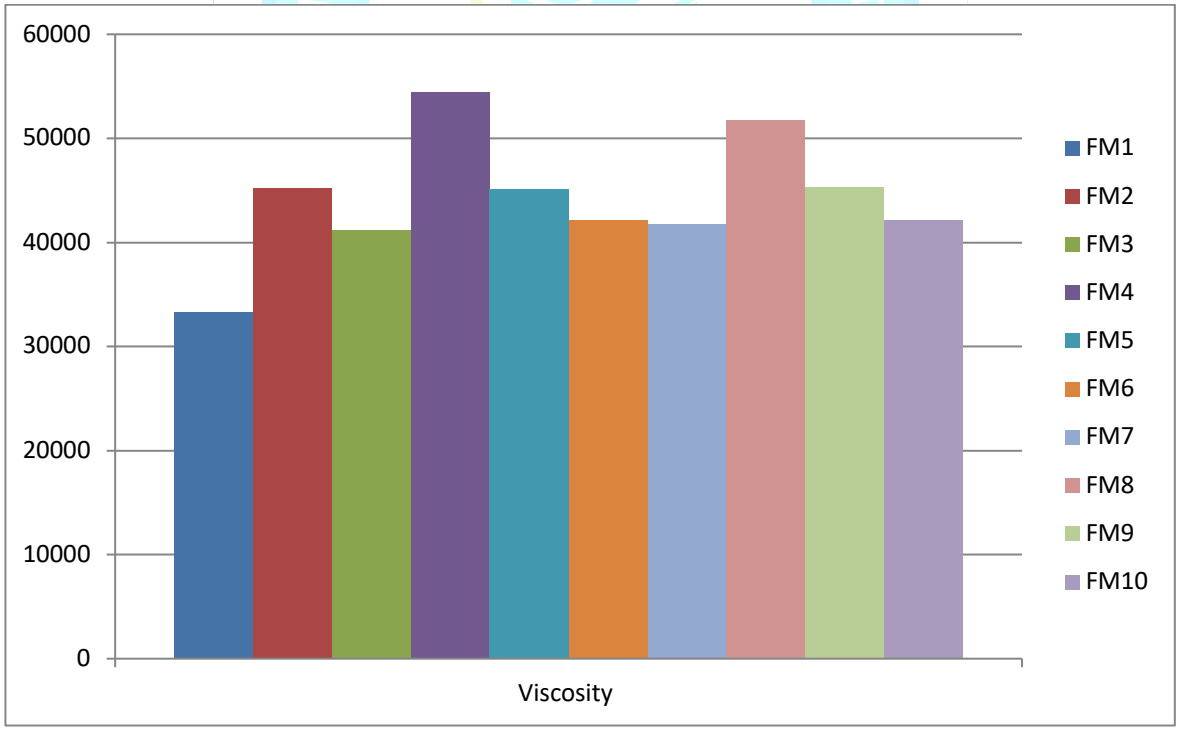

Fig 5: Evaluation parameters for gel (Viscosity)

2.9 Pharmacological Screening

2.9.1 Inhibition of albumin denaturation

The table evaluates the effect of combined methanolic extracts of *Cassia fistula* and *Lawsonia inermis* on albumin denaturation inhibition (% inhibition) at various concentrations (µg/ml). The formulations FM-7 and FM-8 demonstrate significant anti-inflammatory activity in a concentration-dependent manner. Diclofenac (100 µg/ml), used as the standard, shows 1450.08% inhibition. Among the test formulations, FM-2 (100 µg/ml) exhibits 150.05% inhibition, FM-4 (200 µg/ml) shows 165.07%, FM-7 (300 µg/ml) presents 260.06%, and FM-8 (400 µg/ml) achieves the highest inhibition at 315.15%. These results indicate that higher concentrations of the combined extracts exhibit potent protein stabilization, supporting their use in anti-inflammatory topical applications. Among all, FM-8 demonstrated the highest inhibition, indicating a strong ability to stabilize protein structure under stress conditions. The progressive increase in inhibition with concentration confirms the synergistic anti-inflammatory potential of the combined extracts.

Table 9: Effect of Combined drug extract on inhibition of albumin denaturation

S. No	Sample	Concentration (µg/ml)	% Inhibition
1	Control	–	–
2	Diclofenac sodium (Standard)	100	1450.08
3	Gel Formulation FM-2 (*Cassia* + *Lawsonia*)	100	150.05
4	Gel Formulation FM-4 (*Cassia* + *Lawsonia*)	200	165.07
5	Gel Formulation FM-7 (*Cassia* + *Lawsonia*)	300	260.06
6	Gel Formulation FM-8 (*Cassia* + *Lawsonia*)	400	315.15

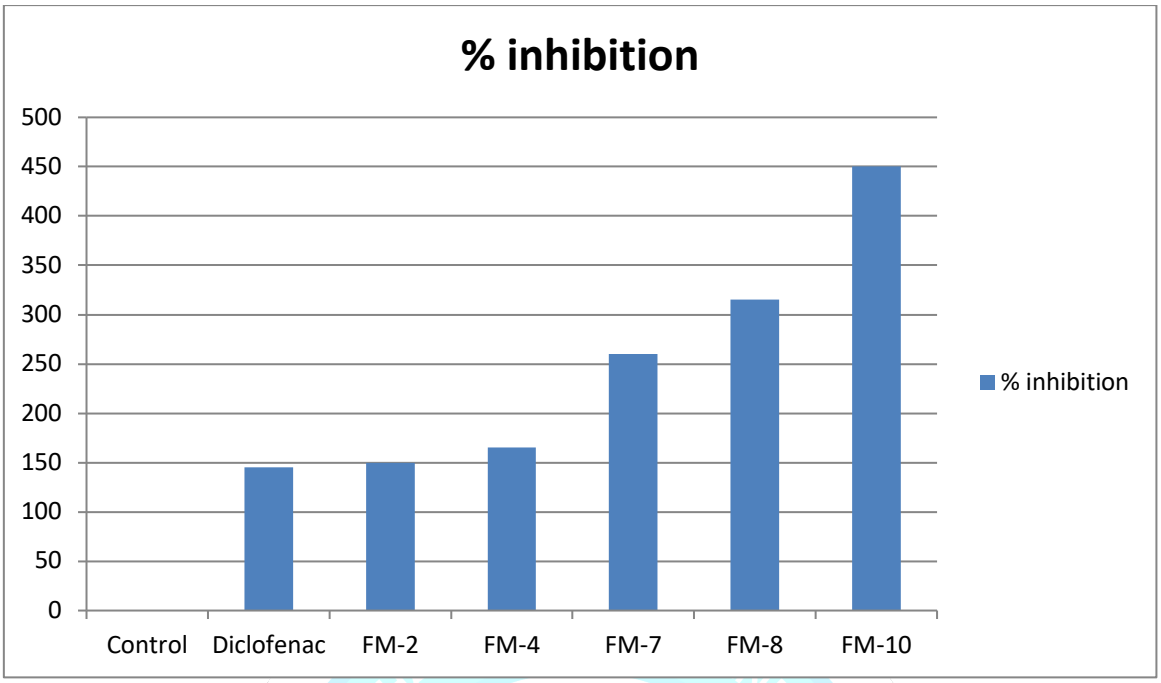

Fig 6: Effect of Combined drug extract on inhibition of albumin denaturation

2.10 In vitro release study

The in vitro drug release of herbal gel formulations containing methanolic extracts of *Cassia fistula* and *Lawsonia inermis* was evaluated using a Franz diffusion cell. A cellophane membrane soaked in phosphate buffer pH 6.8 was mounted between the donor and receptor compartments. A fixed quantity of gel was placed in the donor compartment, while the receptor compartment was filled with buffer and maintained at $37 \pm 0.5°C$ with continuous stirring. Samples were withdrawn at regular intervals and analyzed spectrophotometrically to determine the amount of extract released over time. The release profiles indicated a sustained and concentration-dependent release pattern, with formulations like FM-7 and FM-8 showing enhanced and prolonged release, attributed to the optimal polymer ratio and gelling agents. This confirms the suitability of the gel system for controlled topical delivery of the combined herbal extracts. The release data best fit the Higuchi and Korsmeyer-Peppas kinetic models, suggesting a diffusion-controlled and non-Fickian (anomalous) transport mechanism. Overall, the results confirmed that the gel formulations, particularly FM-7 and FM-8, are suitable for sustained and effective topical delivery of combined herbal extracts with potential anti-inflammatory benefits.

Table 10: In vitro release study of gel containing methanol drug extract

Time (min)	FM-2 (%)	FM-7 (%)	FM-10 (%)
15	20.15	15.20	18.20
30	20.15	16.23	25.80
45	32.15	25.20	30.85
60	42.50	35.30	40.15
90	50.05	45.10	55.03
120	60.80	45.20	62.20
180	75.25	65.30	72.08
240	76.40	70.05	75.08

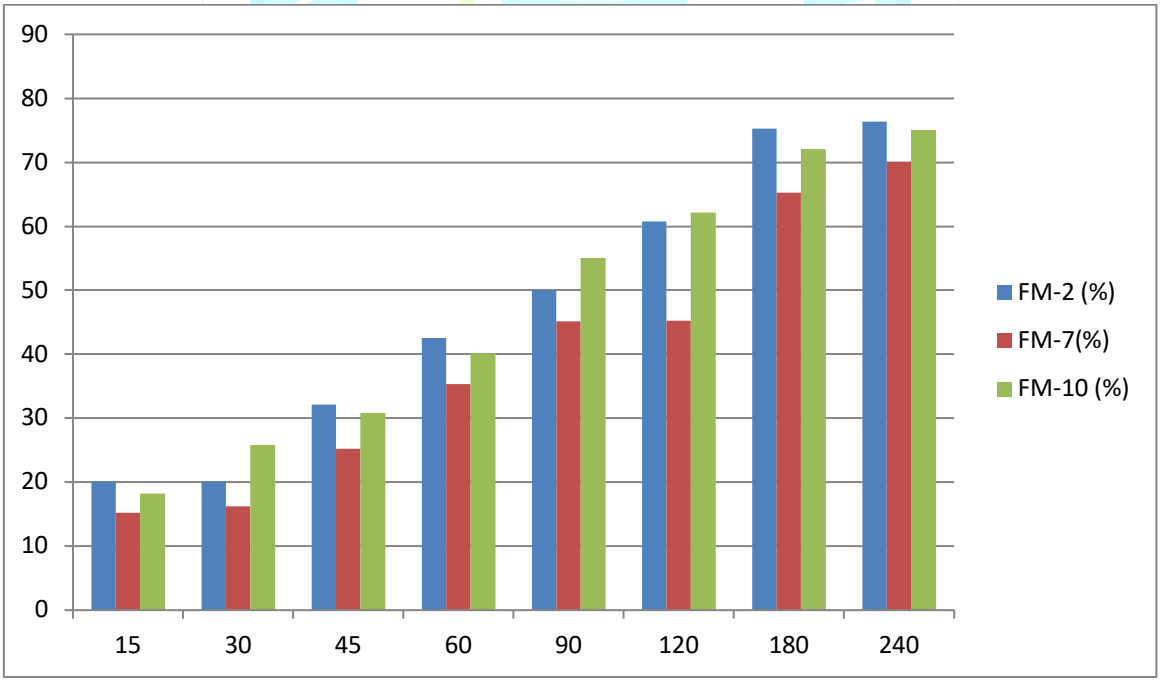

Fig 7: Comparative *in vitro* release study of gel

3. CONCLUSION

The present research highlights the successful development and evaluation of topical herbal gel formulations incorporating methanolic extracts of *Cassia fistula* and *Lawsonia inermis*. Both plants were confirmed through phytochemical screening to be rich in bioactive compounds such as flavonoids, alkaloids, glycosides, and tannins, which contribute to their anti-inflammatory potential. Ten different formulations (FM1–FM10) were prepared using varying gelling agents and concentrations to optimize physicochemical and therapeutic performance. Among them, FM-7 and FM-8 demonstrated superior properties in terms of pH stability, spreadability, homogeneity, and viscosity. The in vitro anti-inflammatory activity, measured by inhibition of albumin denaturation, revealed that FM-8 exhibited the highest percentage inhibition, closely approaching the standard drug diclofenac. Furthermore, in vitro diffusion studies confirmed a sustained release profile, validating the efficiency of the gel matrix in controlled drug delivery. These findings support the potential of *Cassia fistula* and *Lawsonia inermis* in herbal gel formulations as safe and effective alternatives for managing inflammatory skin conditions.

4. Acknowledgment

We express our sincere gratitude to Institution for providing the necessary facilities and resources to conduct this research. We are also grateful to our colleagues and laboratory staff for their assistance in experimental procedures and data analysis.

5. Conflict of Interest

The authors declare no conflict of interest related to this research.

6. References

- Chandra, S., Chatterjee, P., Dey, P., & Bhattacharya, S. (2012). Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. *Asian Pacific Journal of Tropical Biomedicine*, *2*(1), S178–S180. https://doi.org/10.1016/S2221-1691(12)60152-3
- Dash, S., Murthy, P. N., Nath, L., & Chowdhury, P. (2010). Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmaceutica*, *67*(3), 217–223.

- Garg, A., Aggarwal, D., Garg, S., & Singh, R. M. (2002). Spreading of semisolid formulations: An update. *Pharmacologyonline*, *1*, 1–8.
- Indian Pharmacopoeia Commission. (2007). *Indian Pharmacopoeia* (Vol. 2). Ghaziabad: Government of India.
- Jagetia, G. C., & Baliga, M. S. (2004). Evaluation of the anti-inflammatory activity of the leaf extract of *Cassia fistula* L. in rats. *Journal of Ethnopharmacology*, *90*(2–3), 249–252. https://doi.org/10.1016/j.jep.2003.10.005
- Kokate, C. K., Purohit, A. P., & Gokhale, S. B. (2010). *Pharmacognosy* (45th ed.). Pune: Nirali Prakashan.
- Kumar, S., Kumar, D., Deshmukh, R. R., Lokhande, P. D., & More, M. P. (2012). Formulation and evaluation of topical gel of *Lawsonia inermis* for wound healing activity. *International Journal of Pharmaceutical Sciences and Research*, *3*(1), 245–252.
- Kumar, V., Sharma, A., & Kumar, D. (2017). Evaluation of herbal gel formulation of *Lawsonia inermis* leaves for its wound healing activity. *Journal of Pharmacognosy and Phytochemistry*, *6*(3), 442–446.
- Mukherjee, P. K. (2002). *Quality control of herbal drugs: An approach to evaluation of botanicals*. New Delhi: Business Horizons.
- Puranik, S., & Bhise, K. (2012). Formulation and evaluation of herbal gel containing methanolic extract of *Cassia fistula* bark. *International Journal of Research in Ayurveda and Pharmacy*, *3*(6), 805–808.
- Singh, A., & Singh, D. K. (2001). Antifertility activity of *Lawsonia inermis* in male albino rats. *Indian Journal of Experimental Biology*, *39*(7), 682–685.
- Sundaram, R., Nandhakumar, M., & Sasikumar, M. (2011). Antiinflammatory effect of methanolic extract of *Cassia fistula* Linn. bark in rats. *Asian Pacific Journal of Tropical Biomedicine*, *1*(4), 330–333. https://doi.org/10.1016/S2221-1691(11)60071-7