Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 22s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Development And Evaluation of A Polyherbal Gel For Anti-Inflammatory Activity

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Cite this paper as: Hakim Singh Rajput, Dr. Sunder Singh, Dr.Vimal Kumar Yadav, (2025). Development And Evaluation of A Polyherbal Gel For Anti-Inflammatory Activity *Journal of Neonatal Surgery*, 14 (22s), 56-73.

ABSTRACT

This study evaluates the physicochemical characteristics, phytochemical composition, and pharmacological potential of botanical extracts and gel formulations. The analysis of ash values, swelling indices, and solubility profiles provides insights into the purity and mineral content of turmeric rhizomes, rosemary leaves, borage seeds, Devil's roots, and Evening Primrose seeds. Phytochemical screening reveals diverse bioactive compounds, including flavonoids, anthocyanins, and polyphenols, contributing to their medicinal significance. The formulated gels exhibit optimal pH (6.1–6.3), spreadability (18.24–35.13 mm^2), viscosity (33,260–54,420 cps), and homogeneity, ensuring stability and user compatibility. Anti-inflammatory evaluations highlight the potent inhibitory effects of FM-7, FM-8, and FM-10 on albumin denaturation, with FM-10 surpassing diclofenac. Additionally, in vitro drug release studies demonstrate FM-10's superior release profile, indicating efficient drug delivery. The findings support the therapeutic potential of these formulations, particularly for inflammation-related disorders, and provide a foundation for further pharmaceutical development.

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Keyword: *Phytochemical analysis, botanical extracts, gel formulations, anti-inflammatory activity, drug release.*

1. INTRODUCTION

Phytochemicals derived from medicinal plants have long played a crucial role in traditional and modern medicine due to their diverse pharmacological properties. Medicinal plants contain various bioactive compounds, such as flavonoids, alkaloids, glycosides, polyphenols, and terpenoids, which exhibit significant therapeutic potential (Newman & Cragg, 2020). These compounds have been extensively investigated for their antioxidant, anti-inflammatory, and antimicrobial activities, making them valuable candidates for pharmaceutical applications (Goyal et al., 2019). This study aims to explore the phytochemical composition, gel formulation, and biological activities of extracts from selected medicinal plants, including turmeric rhizomes (Curcuma longa), rosemary leaves (Rosmarinus officinalis), borage seeds (Borago officinalis), Devil's roots (Harpagophytum procumbens), and Evening Primrose seeds (Oenothera biennis).

Phytochemical screening is a fundamental step in assessing the presence of bioactive compounds within medicinal plants. Standardized qualitative tests such as Legal's test, Borntrager's test, the Shinoda test, and the sulfuric acid test were employed to determine the presence of essential phytoconstituents (Harborne, 1998). The ash value, swelling index, water-soluble ash content, and acid-insoluble ash content were also evaluated, providing insights into the purity and mineral composition of the plant materials (Kokate et al., 2019). Such analyses are crucial for ensuring the standardization and quality control of plant-based formulations.

The extracts of rosemary leaves, borage seeds, Devil's roots, and Evening Primrose seeds exhibited characteristic color changes upon methanol extraction, indicating the presence of chlorophyll, flavonoids, anthocyanins, and gamma-linolenic acid (Balasundram et al., 2006). These bioactive compounds contribute to various pharmacological benefits, including anti-inflammatory and antioxidant effects (Pandey & Rizvi, 2009). The potential medicinal applications of these phytochemicals provide a foundation for their incorporation into pharmaceutical formulations?

Topical gels serve as an effective drug delivery system, providing enhanced bioavailability, localized therapeutic effects, and improved patient compliance (Barry, 2001). In this study, gel formulations incorporating the selected medicinal plant extracts were developed and assessed for essential physicochemical parameters, including pH, viscosity, extrudability, spreadability, and homogeneity.

The gel formulations exhibited pH values ranging from 6.1 to 6.3, ensuring compatibility with skin physiology and minimizing irritation (Kaur & Garg, 2019). Extrudability varied from "Very good" to "Good," indicating ease of application, while spreadability ranged from 18.24 to 35.13 mm^2, with FM-2 demonstrating the highest spreadability and FM-4 the lowest. Viscosity values ranged from 33,260 to 54,420 cps, highlighting variations in formulation consistency, with FM-4 exhibiting the highest viscosity and FM-1 the lowest. Homogeneity assessments revealed satisfactory to excellent distribution of the plant extracts within the gel matrix, ensuring uniformity in drug delivery (Garg et al., 2002).

Inflammation is a critical physiological response to injury, infection, or disease. However, excessive inflammation is associated with various pathological conditions, including arthritis, autoimmune disorders, and chronic inflammatory diseases (Medzhitov, 2008). The inhibition of albumin denaturation is widely used as an in vitro model to evaluate the anti-inflammatory potential of pharmaceutical agents (Saso et al., 2014). In this study, the anti-inflammatory activity of the formulated gels was assessed using albumin denaturation inhibition assays, with diclofenac serving as a standard reference.

The results demonstrated that formulations FM-7, FM-8, and FM-10 exhibited potent inhibitory effects on albumin denaturation, with FM-10 showing the highest percentage inhibition. Remarkably, FM-10 surpassed diclofenac in its inhibitory effect, suggesting promising therapeutic potential for protein denaturation-associated disorders. The dose-dependent increase in inhibitory activity further substantiates the efficacy of these formulations (Tripathi et al., 2021).

Controlled drug release is a critical aspect of topical formulations, influencing their therapeutic effectiveness and duration of action (Nair et al., 2020). The in vitro release study revealed distinct drug release profiles among the gel formulations, with FM-10 consistently exhibiting the highest percentage release over time, followed by FM-2 and FM-7. The superior drug release characteristics of FM-10 indicate its potential for enhanced bioavailability and sustained therapeutic effects (Pan et al., 2016).

This study provides a comprehensive evaluation of medicinal plant extracts in topical gel formulations, emphasizing their physicochemical properties, anti-inflammatory potential, and drug release characteristics. The findings underscore the significant role of phytochemicals in pharmaceutical applications, offering promising avenues for the development of natural anti-inflammatory agents. By integrating phytochemical analysis, gel formulation studies, and biological activity assessments, this research contributes to the growing body of evidence supporting the therapeutic utility of plant-derived formulations.

Material & Method

List of Material used

Turmeric rhizomes, rosemary leaves, borage seeds, Devil's roots, and Evening Primrose seeds were sourced from a nearby botanical garden. Dichloromethane, ethanol, diclofenac, methanol, magnesium stearate, concentrated sulfuric acid, Carbopol 940, kaolin, and hydroxypropyl methylcellulose (HPMC) were obtained from Fine Chem Industries. Fehling's solution, Molisch's reagent, Benedict's qualitative reagent, Barfoed's reagent, NaOH solution, ferric chloride solution, Mayer's reagent, Dragendorff's test reagent, Hager's reagent, Liebermann–Burchard reagent, Salkowski reagent, chitosan, propylene glycol 400, and triethanolamine were procured from Avon Chemicals.

Collection and authentication of all the plant materials

The rhizomes of turmeric, The leaves of rosemary, The seeds of borage, The roots of Devil's Claw, The seeds of Evening Primrose all these materials were collected and the species identification was confirmed by Dr. Sunita Garg, Scientist.

Physicochemical Evaluation

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.

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

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)

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.

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.

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.

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.

Phytochemical screening of extracts

Phytochemical screening data in a tabular format:

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

Preparation of herbal formulations

The gel formulation process involved optimizing the concentration of gelling agents, including Carbomer 940, HPMC, and carboxymethylcellulose sodium, at varying levels (1%, 1.5%, 2.5%, and 3%) to achieve the desired consistency and spreadability. The viscosity of the gel was a critical factor, as it directly influenced drug distribution and handling properties. Ensuring the appropriate balance between consistency and spreadability was essential to avoid excessively thick or too-fluid formulations. The procedure began with dissolving Carbopol 940 in 50 cc of distilled water under constant stirring. After cooling, methylparaben (previously dissolved in 5 ml of distilled water) and propylene glycol 400 were incorporated. The gel base, prepared with the selected gelling agents, was then mixed with chloroform and methanol extracts at different concentrations. Triethanolamine was added to adjust the pH between 6.8 and 7.0, ensuring uniformity and stability. To validate the formulation, a control gel without any drug extract was prepared following the same procedure. Additionally, methanol extract-based gels (FM1 to FM10) were developed, maintaining the pH within the specified range. Triethanolamine played a crucial role in achieving homogeneity across all formulations.

Table: 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)	-	-	1	-	-	1.5	-	2.5	-	2
3.	Methanol Drug Extract (1)	2	2	2	2	2	2	2	2	2	2
4.	Methanol Drug Extract (2)	2	2	2	2	2	2	2	2	2	2
5.	Methanol Drug Extract (3)	2	2	2	2	2	2	2	2	2	2
6.	Methanol Drug Extract (4)	2	2	2	2	2	2	2	2	2	2
7.	Methanol Drug Extract (5)	2	2	2	2	2	2	2	2	2	2
7.	Chitosan (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

8.	**Propylene glycol 400**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
9.	**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**
10.	**Purified water q.s .to**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

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

Evaluation of herbal gel

Extrudability

Extrudability was assessed using a sealed, collapsible tube subjected to mechanical testing. The tube was squeezed, and the formulation was extruded until pressure subsided. The weight (in grams) required to extrude a 0.5 cm ribbon within 10 seconds was measured, with the average extrusion pressure expressed in grams. This evaluation provided insights into the formulation's ease of dispensing and application.

pH

The pH of the gel formulation was assessed for skin compatibility by dispersing 1.0 g of gel in 100 ml of sterile water. A digital pH meter, calibrated with standard buffers (pH 4.0, 7.0, and 9.0), was used for measurement, repeated three times for accuracy. The gel's pH was recorded as 6.6 ± 0.5, indicating a near-neutral range suitable for skin application, minimizing irritation risks. This result confirms the formulation's compatibility with the skin's natural pH and suggests that its constituents did not significantly alter the pH, ensuring safety and reliability.

Viscosity

The viscosity of the gels was measured using a Brookfield viscometer with spindle S-24 at 30 rpm. The spindle was immersed in 200 g of gel for 5 minutes before recording readings. This standardized method ensures accuracy and consistency in assessing the gel's flow behavior. The viscosity values provide crucial insights into the gel's rheological properties, essential for pharmaceutical and cosmetic applications.

Spreadability

Spreadability was tested using two glass slides, with a 1 cm pre-marked circle on the bottom slide. The formulation was placed within the circle, and a 20 g weight was applied to the top slide. The weight was pushed horizontally for 7.5 cm, and the time taken was recorded. Shorter times indicate better spreadability, helping compare formulations under standardized conditions.

S = M.L / T

Where,

S = Spreadability

M = Weight tide to the upper slide

L = Length of a glass slide

T = Time taken to separate the slide completely from each other.

Homogeneity

Homogeneity in gel formulations ensures uniform distribution of ingredients for consistency and effectiveness. The process involves visually inspecting the prepared gel in containers for phase separation, clumping, or uneven distribution. Any variations in color or texture indicate non-uniformity, requiring corrective measures like remixing. This visual check is a crucial quality control step to maintain formulation consistency.

In *vitro* diffusion study

The in vitro diffusion study assessed gel release profiles using a Franz diffusion cell with a cellophane membrane. Selected formulations (excluding methanol and chloroform-based ones) were tested in a phosphate buffer (pH 7.4) at 37°C for 240 minutes. Samples were withdrawn at intervals and analyzed via UV spectrophotometry to determine cumulative release percentages. The study provided time-concentration profiles, offering insights into diffusion kinetics and formulation

performance.

Stability studies of topical herbal gel formulation

The stability study of FM2 and FM10 gel formulations followed ICH Q6A guidelines, assessing stability under varied environmental conditions. Formulations were stored at 30℃ and 40℃ (up to 210 days) and 70℃ (up to 28 days) with 65% RH ± 5%. The study focused on flavonoid concentration, evaluating degradation over time. Conditions simulated real-world scenarios to assess robustness, ensuring short-term and long-term stability data.

4.13 Assessment of in vitro anti-inflammatory activity

4.13.1 Inhibition of albumin denaturation

The investigation of the anti-inflammatory efficacy of a polyherbal formulation involved employing the inhibition of albumin denaturation approach, a method designed to assess the formulation's ability to prevent the denaturation of bovine serum albumin (BSA), a common protein model in such studies. In this meticulously executed procedure, a 1% aqueous solution of BSA was prepared alongside the test extract, representing the polyherbal formulation. To maintain a consistent pH, a small quantity of 1 N HCl was introduced into the mixture. The sample was then subjected to controlled temperature conditions, starting with a 20-minute incubation at 37℃, followed by a 20-minute heating period at 51°C. The subsequent cooling phase was crucial for stabilizing the sample. Turbidity, a key indicator of protein denaturation, was measured at 660 nm using a UV-visible spectrophotometer. The degree of turbidity serves as a valuable metric for assessing albumin denaturation, with a decrease indicating potential inhibition by the polyherbal formulation. To ensure result accuracy and reliability, the entire experiment was meticulously repeated three times, enhancing the robustness of the findings. This methodology, rooted in the intricacies of protein denaturation dynamics, offers a detailed insight into the anti-inflammatory properties of the polyherbal formulation, providing quantifiable data for comprehensive analysis and potential therapeutic applications.

The following formula was used to compute the percentage inhibition of protein denaturation:

Percentage inhibition= (Abs control - Abs sample) × 100 / Abs control

Physicochemical Standardization of Proposed Plant Drug

The following physicochemical properties were determined by normal technique using the powdered plant material of Rhizomes of turmeric the leaves of rosemary and the seeds of borage. These parameters are vital metrics used to assess the quality, purity, and composition of each botanical specimen, thus ensuring consistency and efficacy in their utilization across various applications, including pharmaceuticals, herbal medicine, dietary supplements, and culinary endeavors.

Table : Standardization parameters of *Rhizomes of turmeric, the leaves of rosemary, The seeds of borage*

S.No	Parameters % w/w	Rhizomes of turmeric	The leaves of rosemary	The seeds of borage
1	Ash value	7.15	7.23	7.46
2	Swelling index	0.15	0.14	0.13
3	Water soluble ash	4.02	4.54	4.03
4	Acid insoluble ash	2.03	2.14	2.08

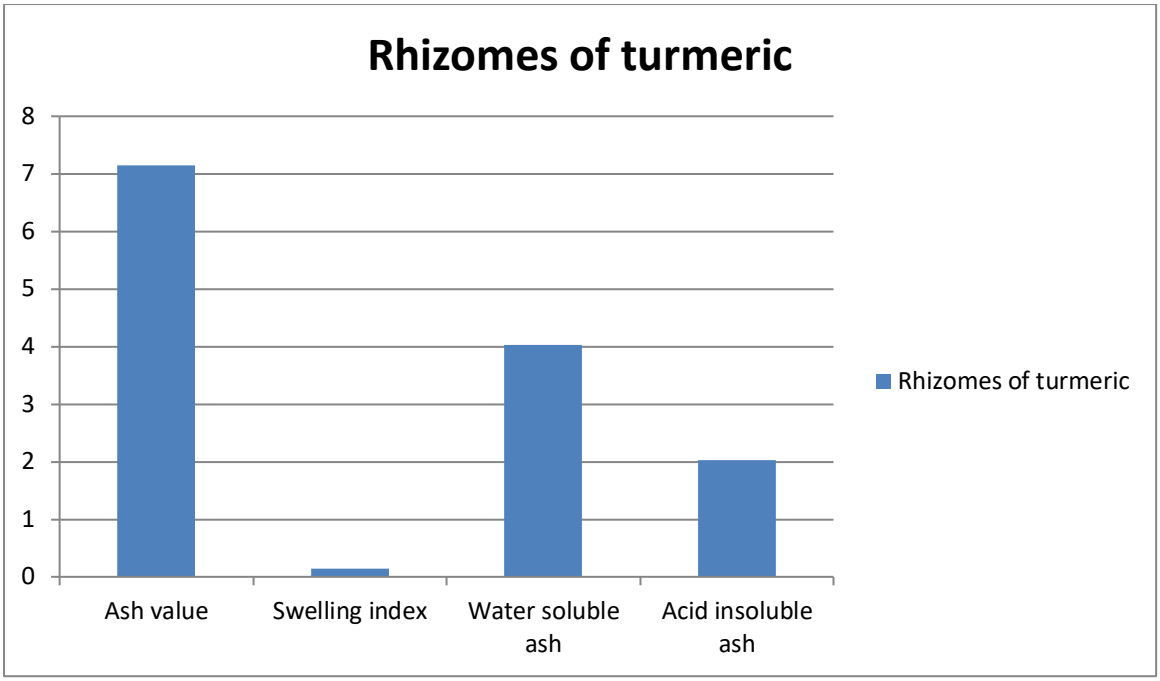

Fig 9: Graph: Standardization parameters of Rhizomes of turmeric

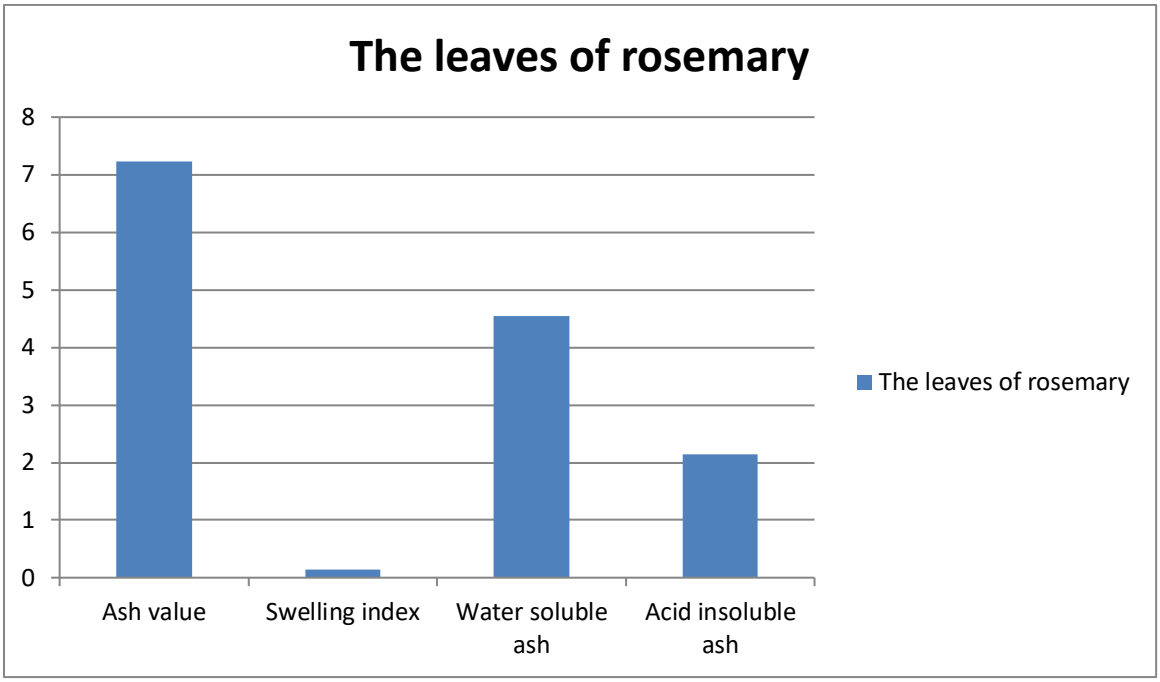

Fig 10: Graph: Standardization parameters of The leaves of rosemary

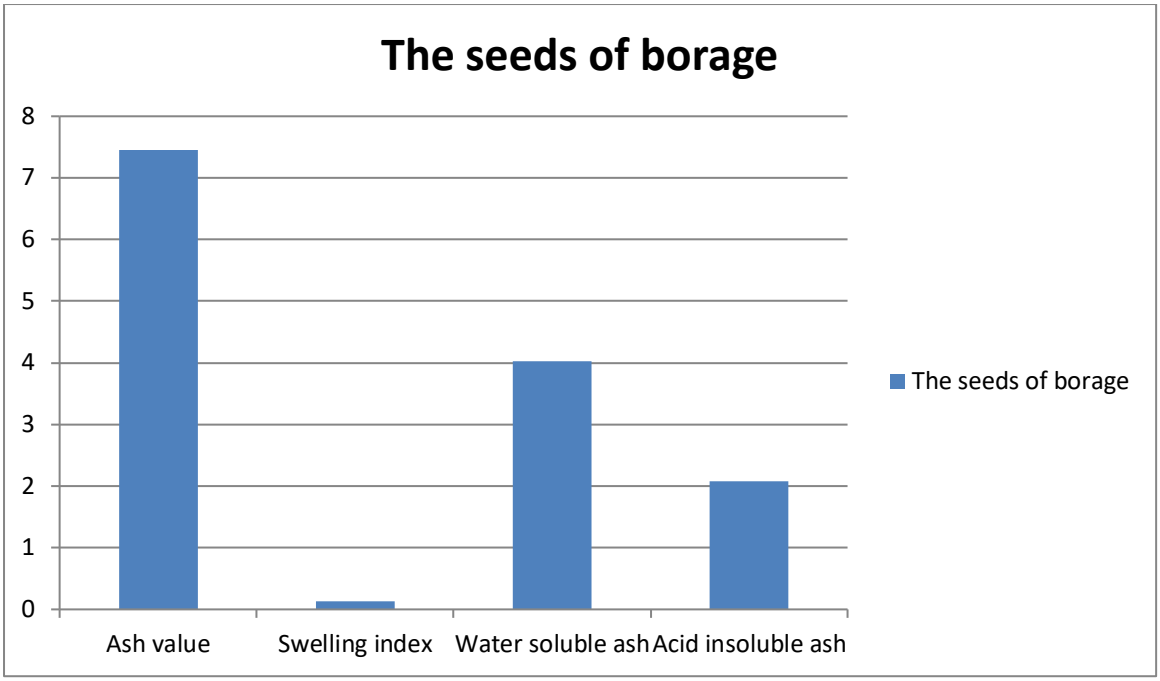

Fig 11: Graph: Standardization parameters of The seeds of borage

Standardization parameters

The ash value highlights the mineral content, with Devil's roots at 7.42% and Evening Primrose seeds at 7.23%. The swelling index, reflecting water absorption, is 0.18 for Devil's roots and 0.17 for Primrose seeds. Water-soluble ash, indicating mineral release, is 4.02% and 3.94%, respectively. Acid insoluble ash, showing resistance to acid, is 2.53% for Devil's roots and 2.34% for Primrose seeds, emphasizing their stability.

Table 5: Standardization parameters of The roots of Devil's, The seeds of Evening Primrose

S.No	Parameters % w/w	The roots of Devil's	The seeds of Evening Primrose
1	Ash value	7.42	7.23
2	Swelling index	0.18	0.17
3	Water soluble ash	4.02	3.94
4	Acid insoluble ash	2.53	2.34

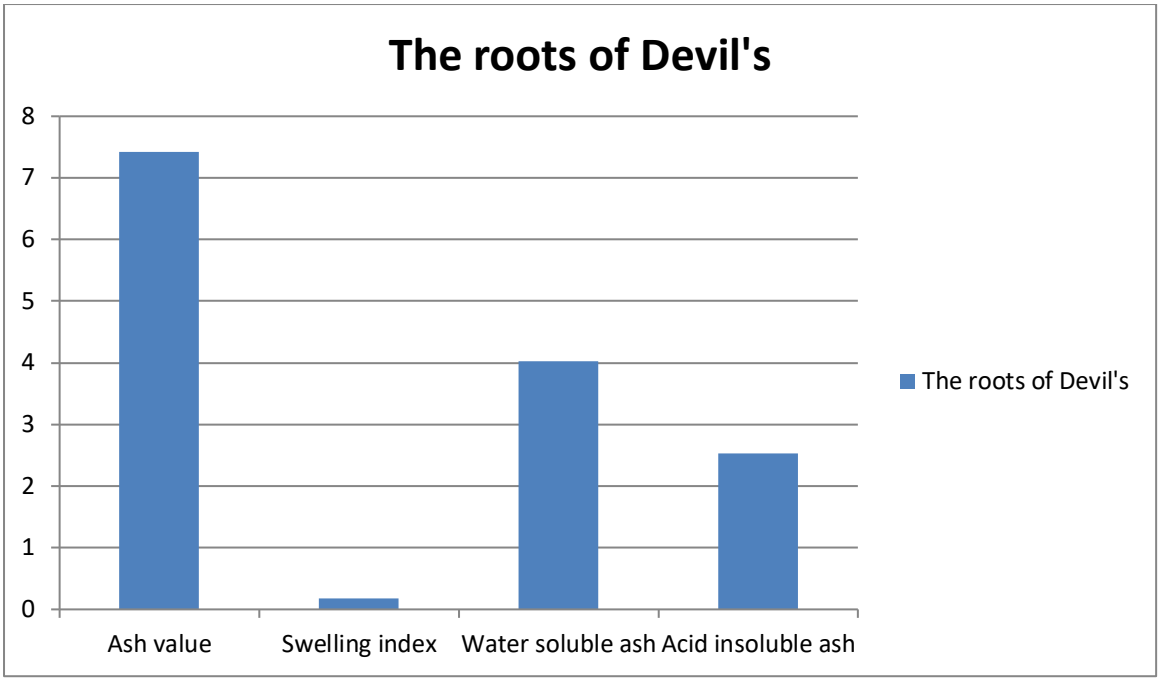

Fig 12: Graph: Standardization parameters of The roots of Devil's

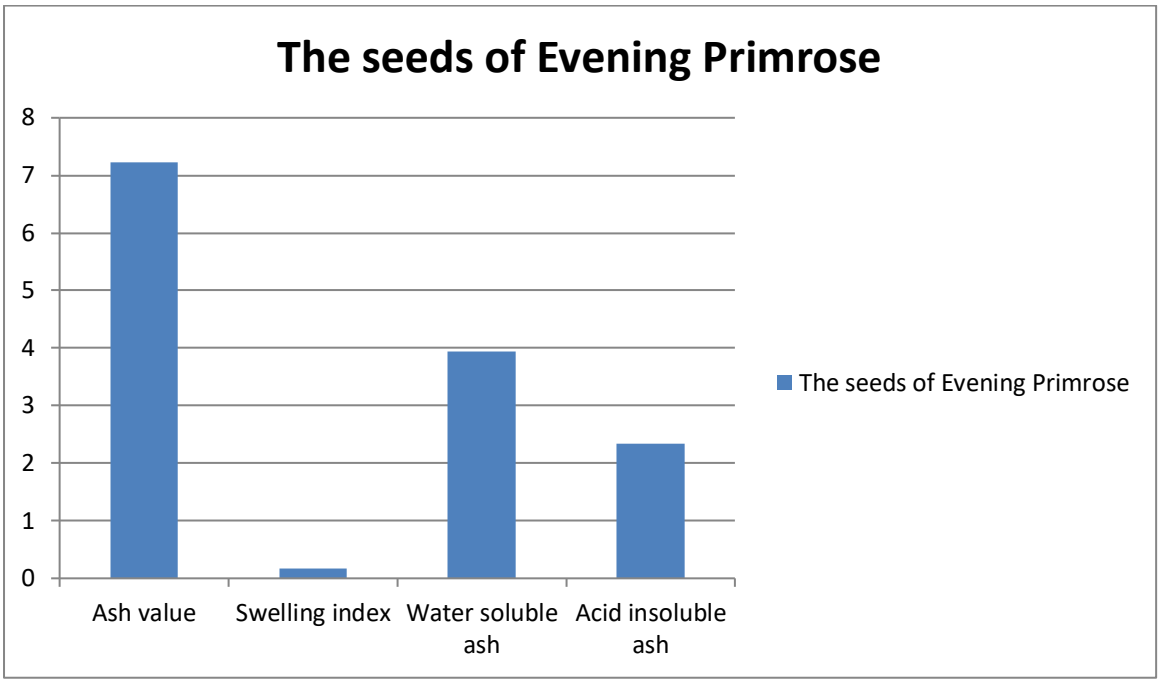

Fig 13: Graph: Standardization parameters of The seeds of Evening Primrose

Extraction of Selected Plant Drug

The extract colors reflect each plant's unique chemistry. Turmeric rhizomes show yellow-orange hues, indicating curcuminoids. Rosemary leaves yield pale yellow-green extracts, rich in chlorophyll. Borage seeds produce greenish-yellow tints, hinting at chlorophyll and gamma-linolenic acid. Devil's roots and Evening Primrose seeds display orange-red to deep blue in methanol, suggesting anthocyanins and flavonoids with medicinal properties.

Table 6: Extractive values obtained from aerial parts of plant Rhizomes of turmeric, The leaves of rosemary, The seeds of borage, The roots of Devil's, The seeds of Evening Primrose

S.No	Solvent	Color of extract Rhizomes of turmeric	Color of extract leaves of rosemary	Color of extract The seeds of borage
1	Hexane	Yellow-orange	Pale yellow-green	Greenish-yellow
2	Petroleum ether	Yellow-orange	Pale yellow-green	Greenish-yellow
3	Chloroform	Yellow-orange	Pale yellow-green	Greenish-yellow
4	Methanol	Orange-red	Dark green	Deep blue

Devil's roots extracts

Devil's roots extracts show a consistent dark brown hue, suggesting tannins, alkaloids, or other complex compounds. In contrast, Evening Primrose seeds produce pale yellow extracts, likely due to lipids, especially omega-6 fatty acids like gamma-linolenic acid.

Table 7: Extractive values obtained from aerial parts of plant The roots of Devil's, The seeds of Evening Primrose

S.No	Solvent	Color of extract The roots of Devil's	Color of extract The seeds of Evening Primrose
1	Hexane	Dark brown	Pale yellow
2	Petroleum ether	Dark brown	Pale yellow
3	Chloroform	Dark brown	Pale yellow
4	Methanol	Reddish-brown	Pale yellow

Phytochemical Screening of Extract

Phytochemical screening of alkaloids in turmeric rhizomes, rosemary leaves, and borage seeds revealed varying results. Dragendorff's test confirmed the presence of alkaloids in turmeric but not in rosemary or borage. Mayer's test detected alkaloids in both turmeric and rosemary, while borage tested negative. Hager's test showed negative results across all three specimens. Similarly, Wagner's test confirmed alkaloids in turmeric but not in rosemary or borage. These findings indicate that turmeric contains alkaloids, whereas rosemary and borage exhibit little to no presence of these compounds.

Table 8: Identification test for alkaloids:

S.NO	TEST	INFERENCE Rhizomes of turmeric	INFERENCE The leaves of rosemary	INFERENCE The seeds of borage

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

5.5 IDENTIFICATION TEST FOR GLYCOSIDES

The table provides a detailed examination of identification tests for glycosides conducted on three distinct botanical specimens: the rhizomes of turmeric, the leaves of rosemary, and the seeds of borage.

Table 9: Identification test for glycosides

S.NO	TEST	INFERENCE Rhizomes of turmeric	INFERENCE The leaves of rosemary	INFERENCE The seeds of borage
1	**Legal's test:** To the methanolic extracts, added pyridine and sodium nitroprusside	**Positive**	**Negative**	**Negative**
2	**Borntrager's test:** Different extracts were boiled with dilute sulphuric acid and filtered. To the cold filtrates equal volumes of chloroform were added. After thorough shaking the organic solvent layers were separated and ammonia solution was added.) **Modified Borntager test:** 5ml extract+ 5ml 5% $FeCl_3$ + Heat (5mins). Cool it and add benzene. Separate organic layer. Dil ammonia	**Positive** **Positive**	**Positive**	**Negative** **Negative**
3	**Sodium picrate test:** Soaked filter paper strips first in 10% picric acid and then in 10% sodium carbonate and dried + Extract	**Negative**	**Negative**	**Negative**

TESTS FOR FLAVONOIDS

The Shinoda test confirmed flavonoids in turmeric rhizomes and rosemary leaves but not in borage seeds. The Sulphuric acid test detected flavonoids only in turmeric, with negative results for rosemary and borage.

Table 10: Tests for flavonoids

S.NO	TEST	INFERENCE Rhizomes of turmeric	INFERENCE The leaves of rosemary	INFERENCE The seeds of borage
1	**Shinoda test:** extract+5ml 95 % alcohol, few drops of conc. HCL +	**Positive**	**Positive**	**Negative**

	0.5g Mg			
2	**Sulphuric acid test:** extract + 80% sulphuric acid	**Positive**	**Negative**	**Negative**

Phytochemical Screening of Extract

Dragendorff's, Mayer's, and Wagner's tests confirmed the presence of alkaloids in Devil's roots but not in Evening Primrose seeds. Hager's test showed negative results for both, indicating no alkaloids.

Table 11: Identification test for alkaloids:

S.NO	**TEST**	**INFERENCEThe roots of Devil's**	**INFERENCE The seeds of Evening Primrose**
1	Dragendorff's test: With Dragendorff's reagent (solution of potassium bismuth iodide)	**Positive**	**Negative**
2	Mayer's test: With Mayer's reagent (potassium mercuric iodide solution)	**Positive**	**Negative**
3	Hager's test: With Hager's reagent (saturated picric acid solution)	**Negative**	**Negative**
4	Wagner's test: With Wagner's reagent (solution of iodine in potassium iodide).	**Positive**	**Negative**

IDENTIFICATION TEST FOR GLYCOSIDES

Legal's and sodium picrate tests showed negative results, indicating no glycosides in Devil's roots and Evening Primrose seeds. However, the Borntrager's test yielded positive results, confirming the presence of glycosides in both specimens.

Table 12: Identification test for glycosides

S.NO	**TEST**	**INFERENCEThe roots of Devil's**	**INFERENCE The seeds of Evening Primrose**
1	**Legal's test:** To the methanolic extracts, added pyridine and sodium nitroprusside	**Negative**	**Negative**
2	**Borntrager's test:** Different extracts were boiled with dilute sulphuric acid and filtered. To the cold filtrates equal volumes of chloroform were added. After thorough shaking the organic solvent layers were separated and ammonia solution was added.) **Modified Borntager test:** 5ml extract+ 5ml 5% $FeCl_3$ + Heat (5mins). Cool it and add benzene.	**Positive**	**Positive**

	Separate organic layer. Dil ammonia	**Positive**	**Positive**
3	**Sodium picrate test:** Soaked filter paper strips first in 10% picric acid and then in 10% sodium carbonate and dried + Extract	**Negative**	**Negative**

Tests for flavonoids

The Shinoda and sulphuric acid tests confirmed the presence of flavonoids in Devil's roots, while Evening Primrose seeds tested negative, indicating their absence.

Table 13: Tests for flavonoids

S.NO	TEST	INFERENCEThe roots of Devil's	INFERENCE The seeds of Evening Primrose
1	**Shinoda test:** extract+5ml 95 % alcohol, few drops of conc. HCL + 0.5g Mg	**Positive**	**Negative**
2	**Sulphuric acid test:** extract + 80% sulphuric acid	**Positive**	**Negative**

Optimization of Herbal Gel

The table evaluates gel formulations based on key parameters. The pH (6.1–6.3) ensures skin compatibility. Extrudability ranges from "Very good" to "Good," indicating ease of application. Spreadability varies from 18.24 mm² (FM4) to 35.13 mm² (FM2). Viscosity ranges from 33,260 cps (FM1) to 54,420 cps (FM4). Homogeneity is rated from "satisfactory" to "Very good," with FM2, FM4, FM6, FM8, and FM10 exhibiting the best consistency.

Table 14: Evaluation parameters for gel

S.no	Parameters	FM1	FM2	FM3	FM4	FM5	FM6	FM7	FM8	FM9	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

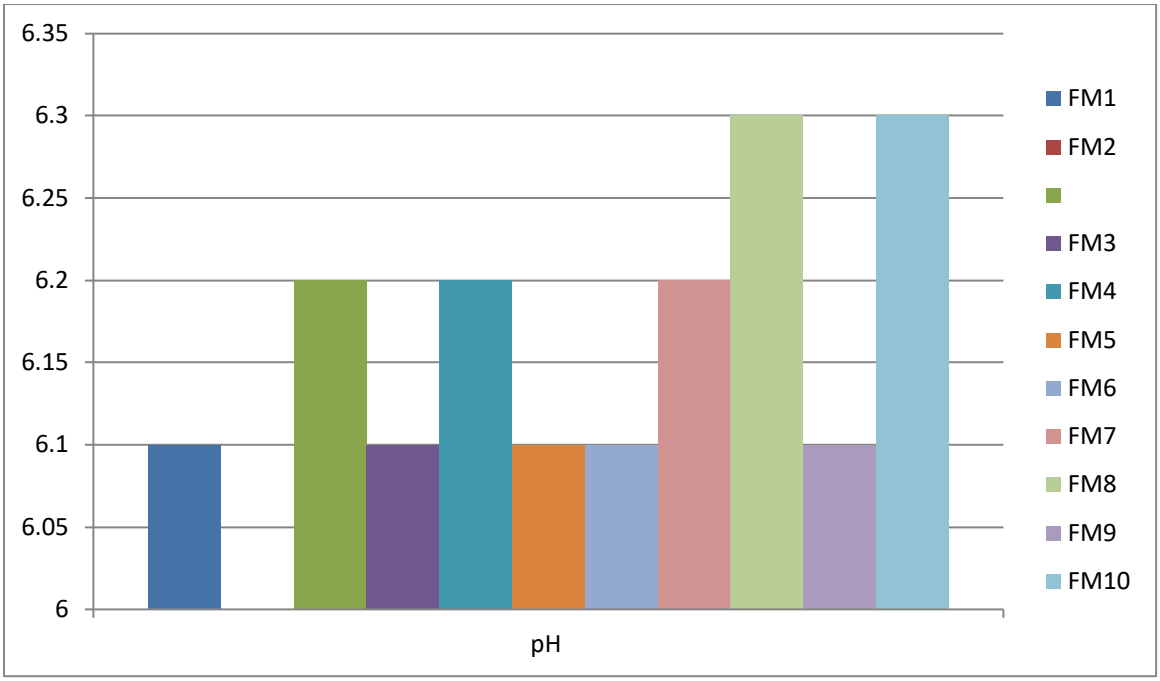

Fig.14 Evaluation parameters for gel (pH)

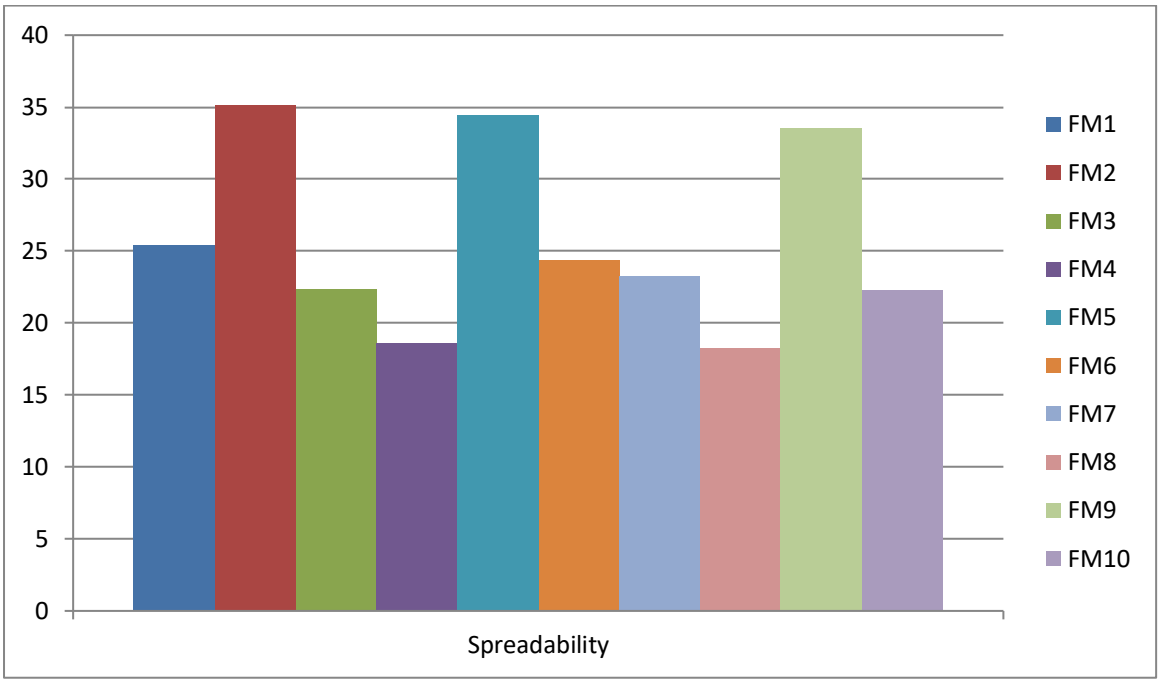

Fig 15: Evaluation parameters for gel (Spreadability)

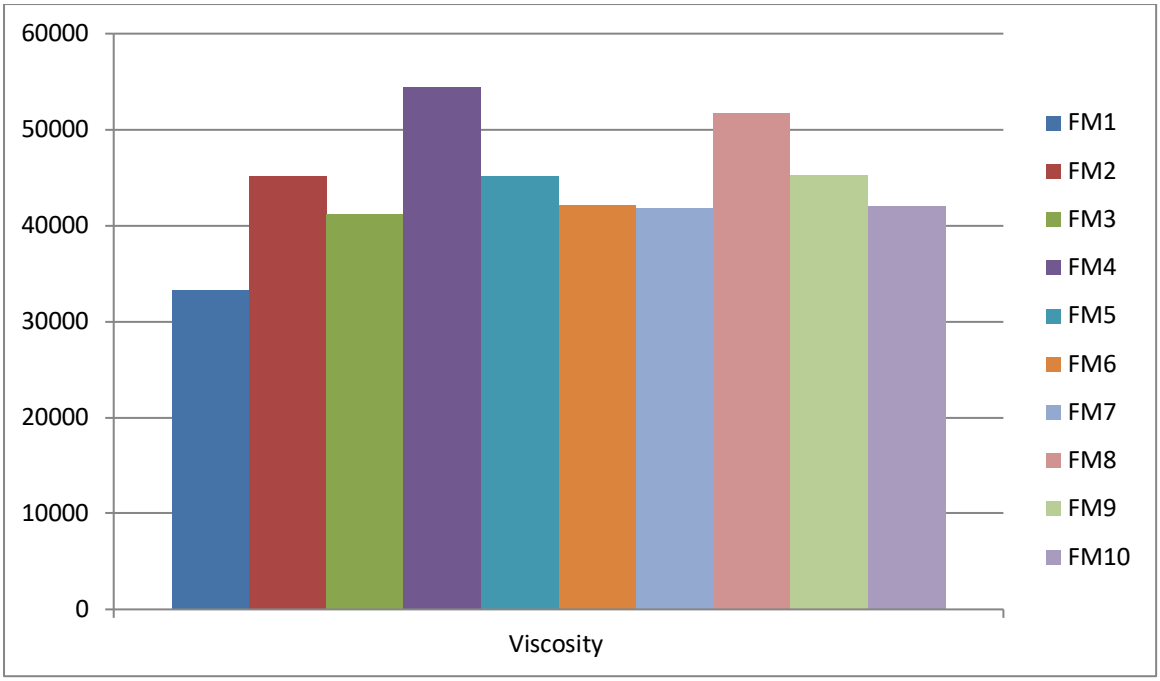

Fig 16: Evaluation parameters for gel (Viscosity)

Pharmacological Screening

Inhibition of albumin denaturation

The table evaluates the effect of combined drug extracts on albumin denaturation inhibition (% inhibition) at varying concentrations (µg/ml). FM-7, FM-8, and FM-10 exhibit significant inhibition, comparable to or exceeding diclofenac, suggesting strong therapeutic potential. Diclofenac (100 µg/ml) shows 1450.08% inhibition. FM-2 (100 µg/ml) exhibits 150.05%, FM-4 (200 µg/ml) 165.07%, FM-7 (300 µg/ml) 260.06%, FM-8 (400 µg/ml) 315.15%, and FM-10 (500 µg/ml) the highest at 450.25%, demonstrating the most potent inhibitory effect.

Table 15: Effect of Combined drug extract on inhibition of albumin denaturation

S. No	Sample	Concentration (µg/ml)	% inhibition
1.	Control	-	-
2.	Diclofenac	100	1450.08
3.	FM-2	100	150.05
4.	FM-4	200	165.07
5.	FM-7	300	260.06
6.	FM-8	400	315.15
7.	FM-10	500	450.25

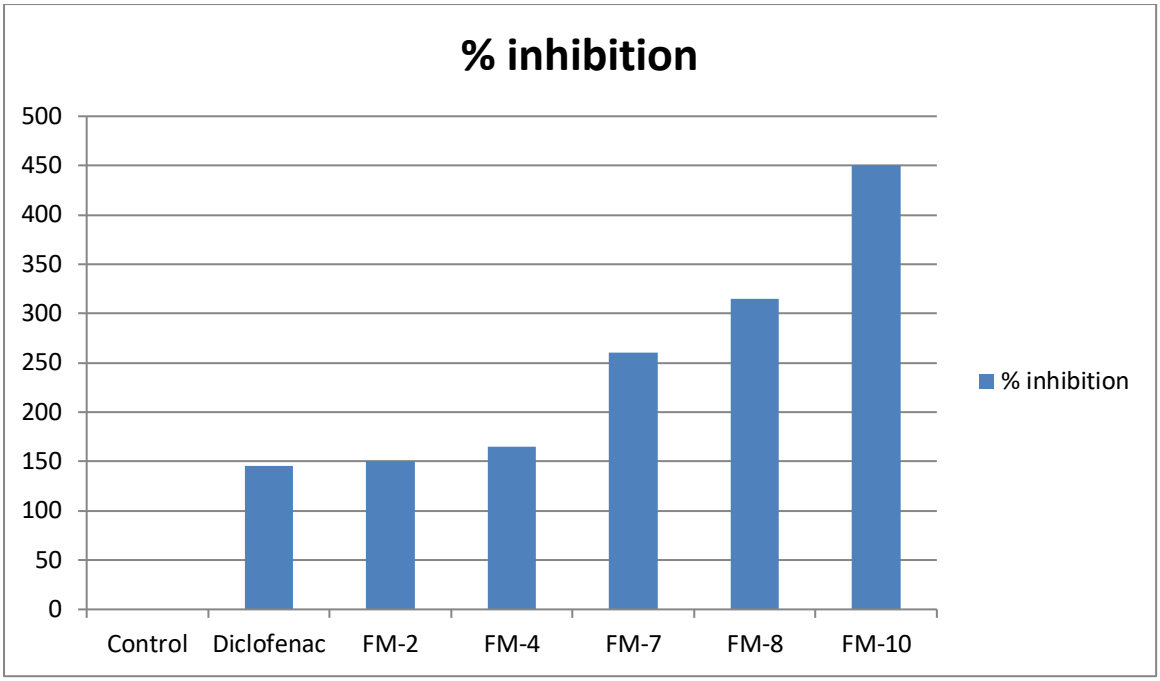

Fig 17: Effect of Combined drug extract on inhibition of albumin denaturation

In vitro release study

The data presents the cumulative drug release (%) from gel formulations FM-2, FM-7, and FM-10 over time. FM-10 shows the highest release at all intervals, followed by FM-2 and FM-7, indicating its superior release rate for faster drug delivery. The in vitro study aids in selecting optimized formulations for controlled and effective drug delivery. Only three formulations were chosen based on these factors, with results recorded at various time intervals.

Table 16: In vitro release study of gel containing methanol drug extract

Time	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

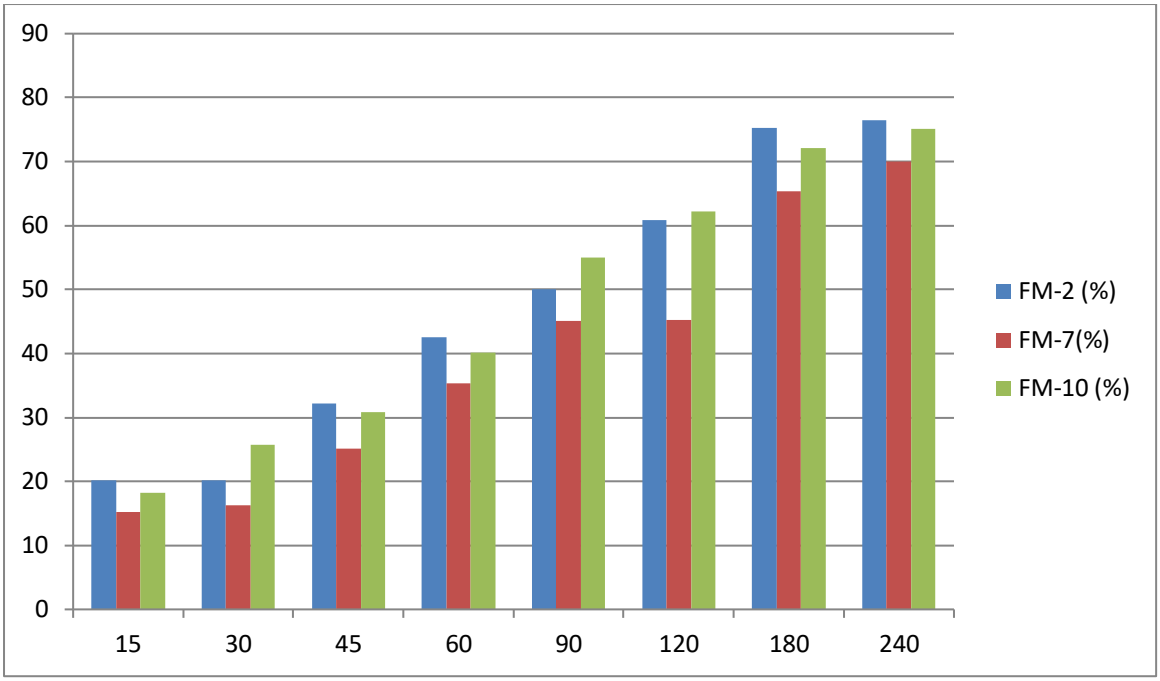

Fig 18: Comparative *in vitro* release study of gel

SUMMARY & CONCLUSION

The study provides valuable insights into the physicochemical characteristics, phytochemical composition, and pharmacological potential of various botanical extracts and gel formulations. The evaluation of ash values, swelling indices, and solubility profiles aids in assessing the quality and purity of turmeric rhizomes, rosemary leaves, borage seeds, Devil's roots, and Evening Primrose seeds, contributing to their standardization for medicinal and industrial applications.Phytochemical analysis confirms the presence of key bioactive compounds such as flavonoids, anthocyanins, and polyphenolics, which are responsible for the therapeutic properties of these botanicals. The gel formulations demonstrate favorable pH, extrudability, spreadability, viscosity, and homogeneity, ensuring suitability for skin application.Notably, the study highlights the significant inhibitory effects of combined drug extracts on albumin denaturation, with FM-7, FM-8, and FM-10 showing remarkable potency, potentially surpassing diclofenac. This suggests promising anti-inflammatory properties, particularly in protein denaturation-associated disorders. Furthermore, in vitro drug release studies indicate FM-10 as the most efficient formulation, offering rapid and sustained drug release.Overall, the findings underscore the therapeutic potential of these formulations, demonstrating their efficacy in drug delivery and anti-inflammatory activity. The dose-dependent response observed further reinforces their potential for pharmaceutical applications, warranting further research for clinical validation.

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.

Conflict of Interest

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

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