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Formulation, Comparative Evaluation, and Stability Assessment of Multiple Topical Gel Formulations Containing Allium sativum and Trigonella foenum-graecum Seed Extracts for Enhanced Anti-inflammatory Efficacy

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ABSTRACT

This investigation focused on the development and comparative analysis of nine topical gel formulations incorporating aqueous extracts of Allium sativum (garlic) and Trigonella foenum-graecum (fenugreek) seeds, aimed at enhancing anti-inflammatory properties. The gels were systematically evaluated through an array of tests including physicochemical characterization, phytochemical profiling, rheological behaviour, and stability assessments. Additionally, in vitro assays for antioxidant, anti-inflammatory, and antifungal effects were conducted. Among the formulations, the combined gel FG3 demonstrated superior synergistic effects, exhibiting enhanced anti-inflammatory efficacy, optimized rheology, and improved storage stability compared to formulations containing individual extracts or commercial products. These results endorse the potential of combined botanical extracts for the formulation of effective topical therapeutics targeting inflammatory skin conditions.

Keywords: *Allium sativum, Trigonella foenum-graecum, topical gel, anti-inflammatory, stability, synergistic formulation.*

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1. INTRODUCTION

Topical herbal formulations have gained prominence in dermatological therapy due to their notable efficacy, safety profile, and acceptance stemming from traditional use. Both Allium sativum and Trigonella foenum-graecum have a well-documented ethnomedicinal history linked to their bioactive constituents such as flavonoids, saponins, and diosgenin. The concurrent use of these extracts holds promise for enhanced therapeutic activity through synergistic interactions, particularly in terms of anti-inflammatory and antimicrobial efficacy. Despite numerous individual evaluations, comprehensive comparative studies focusing on combined extract gels and their long-term stability are limited. This study aims to address this gap by developing multiple topical gel formulations combining these extracts, followed by detailed characterization, performance evaluation, and stability testing for potential clinical application.

Recent advancements in topical gel technology exploit benefits such as ease of application, minimal systemic absorption, and enhanced drug bioavailability compared to ointments. Gels are preferred due to their non-greasy nature, favourable sensory properties, and better patient compliance. Previous research has optimized gels based on single herbs for anti-inflammatory use; however, understanding the combined effects and ensuring pharmaceutical stability remain unmet needs. This work seeks to formulate and assess gels integrating Allium sativum and Trigonella foenum-graecum aqueous extracts, focusing on their anti-inflammatory, antifungal activities, physicochemical properties, patient safety, and storage stability.

2. MATERIALS AND METHODS

Plant Material Collection and Authentication

The garlic cloves (Allium sativum) and fenugreek seeds (Trigonella foenum-graecum) utilized in this study were sourced locally from vendors in Lucknow, India. Authentication was performed by experts at the National Botanical Research Institute, Lucknow, with voucher specimens preserved for future reference

Extraction

Garlic cloves were crushed and extracted using a Soxhlet apparatus with distilled water over a 24-hour period. The extract was then filtered, concentrated, and subjected to lyophilization below 50°C to preserve active constituents. Fenugreek seeds

were similarly crushed and percolated in water containing chloroform as a preservative for 72 hours. The mucilage from the seeds was separated by squeezing and collected for formulation use.

Phytochemical Evaluation

Qualitative assays were conducted to detect the presence of alkaloids, saponins, sterols, glycosides, tannins, flavonoids, proteins, and amino acids using standard chemical methods. Quantitative evaluation included the Folin-Ciocalteu assay for total phenolic content, aluminium chloride colorimetric assay for flavonoids, potassium ferricyanide method for reducing power, DPPH radical scavenging for antioxidant capacity, and UV/TLC-based diosgenin quantification

Topical Gel Formulation

Total Nine formulations are made using Garlic, Fenugreek seed extracts or both with different compositions of excipients. F is Stands for formulation with Fenugreek seed extracts and G is stands for Garlic Extracts and FG stands for both Fenugreek-Garlic extract

Ingredient	**F1**	**F2**	**F3**	**G1**	**G2**	**G3**	**FG1**	**FG2**	**FG3**
DRUG	1	1	1	1	1	1	1	1	1
Pluronic F-68	15	20	25	15	20	25	15	20	25
Carbopol 934	-	0.4	0.8	-	0.4	0.8	-	0.4	0.8
Sodium Alginate	-	0.2	0.4	-	0.2	0.4	-	0.2	0.4
Propylene glycol	1	1	1	1	1	1	1	1	1
Methyl paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Water	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs

Table1-Formulation of Various Topical Gels

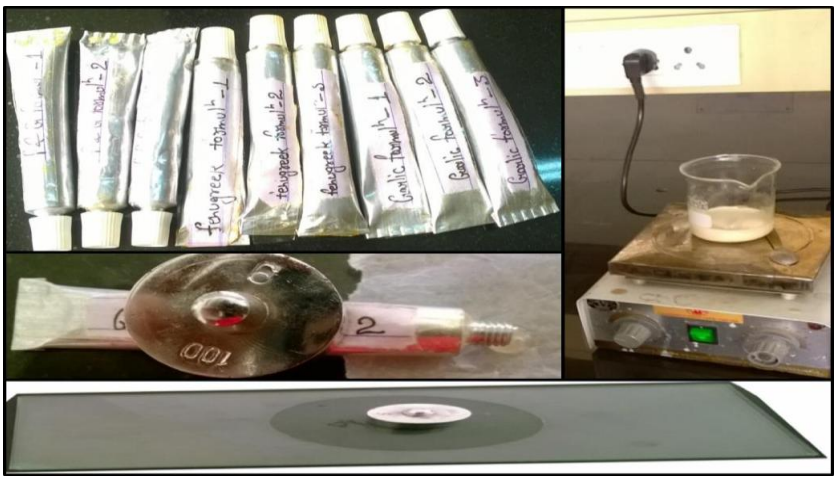


Fig1-Development of various formulations and its evaluation

- Polymers: Carbopol 934, Poloxamer 188, Sodium Alginate (varied across F1–F3, G1–G3, FG1–FG3 gels).
- Gels were prepared by dispersing extracts in hydrated polymer bases with propylene glycol and methyl paraben as solvent/co-solvent and preservative, respectively. pH adjusted to 6.3–6.8

3. EVALUATION OF TOPICAL GELS

The prepared topical formulations were inspected visually for clarity color and presence of any particle.

Appearance: All the prepared formulations were visually examined for clarity, color, and the presence of particulate matter, ensuring uniformity and homogeneity as per standard evaluation protocols.

pH: The pH of each gel formulation was measured using a calibrated digital pH meter. For this, 2.5 g of gel was accurately weighed, dispersed in 25 ml of distilled water, and allowed to stand for two hours. After equilibration, pH readings of each formulation were recorded

Skin Irritation: Dermal irritation testing was carried out on healthy human volunteers (both male and female). Around 100 mg of each gel formulation was applied to a 2 cm^2 region on the inner upper arm, covered with a cotton pad, and left for 6 hours. Observations were made based on the Draize scoring scale: 0 indicated no irritation, 1 slight irritation, and 2 moderate irritation.

Spreadability: Spreadability was evaluated by sandwiching 2 g of gel between two glass slides and applying a 1 kg weight for 5 minutes to achieve a thin uniform layer. The upper slide was then pulled with an 80 g weight, and the time required for it to move 7.5 cm was recorded. Spreadability (S) was calculated using the formula:

Formula: S = M × L / T

Where S = Spreadability

M = Weight in the pan (tied to the upper slide)

L = Length moved by the glass slide

T = Time (in sec.) taken to separate the upper slide from the ground slide.

Extrudability: Extrudability was assessed by extruding the gel from lacquered aluminum collapsible tubes under standard force conditions. The quantity of gel extruded within 10 seconds, forming at least a 0.5 cm ribbon, was noted. The study was conducted in triplicate, and the mean values were reported. Extrudability was determined using the expression:The extrudability was than calculated by using the following formula:

Extrudability = Weight Applied to extrude gel from tube (in gm) / Area (in cm2)

Viscosity: Viscosity of the formulations was measured using a Brookfield viscometer (spindle no. 63) at 100 rpm. Data were expressed in Pascal seconds (Pa·s) or poise. All experiments were performed in triplicate to ensure reliability.

Drug Content: For drug content estimation, 100 mg of gel (formulated and marketed) was separately dissolved in 100 ml of phosphate buffer (pH 7.4) under continuous stirring until complete solubilization. The solutions were filtered, and drug concentrations were determined by UV spectrophotometry at 274 nm, using phosphate buffer (pH 7.4) as the blank.

Rheograms: Viscosity readings were taken at progressively increasing spindle speeds (rpm), followed by a decreasing order of rpm. The results were plotted to generate rheograms, which helped in understanding the flow characteristics and thixotropic behavior of the gels.

Formulation	Appearance	pH	Skin irritation	Viscosity
F1	Light Yellow (Transparent)	6.5	0	2479
F2	Light Yellow (Transparent)	6.8	0	2579
F3	Light Yellow (Transparent)	6.7	0	2750
G	Light Yellow (Transparent)	6.3	0	3467
G2	Light Yellow (Transparent)	6.5	0	3950
G3	Light Yellow (Transparent)	6.6	0	4287
FG1	Light Yellow (Transparent)	6.4	0	2487
FG2	Light Yellow (Transparent)	6.8	0	3257

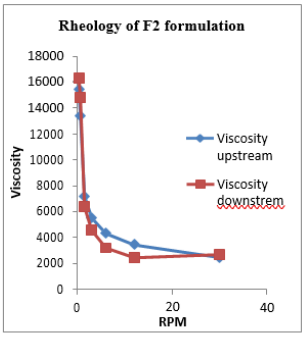
FG3	Light Yellow (Transparent)	6.7	0	3715
Marketed gel	White	6.8	0	3625

Table 2-Evaluation of Appearance, pH, Skin Irritation and Viscosity

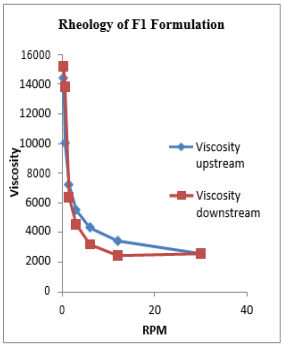
Formulations	Spreadability	Extrudability	Drug Content
F1	19	17.12	47.09
F2	25	18.08	47.81
F3	31	18.39	59.45
G1	20	17.39	41.81
G2	27	18.02	49.27
G3	29	17.83	60.54
FG1	21	18.05	49.72
FG2	26	18.11	53.09
FG3	30	18.79	62.27
Marketed gel	32	19	63.45

Table 3-Evaluation of Spreadability, Extrudability, and Drug Content

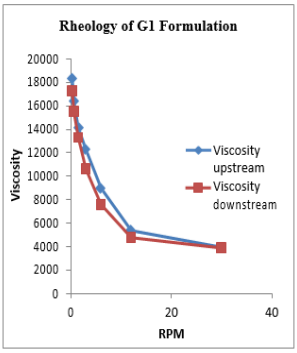
RPM	Viscosity upstream	Viscosity down stream
0.3	15397	16297
0.6	13355	14798
1.5	7198	6358
3	5519	4559
6	4319	3200
12	3439	2449
30	2479	2679
60	EE	EE



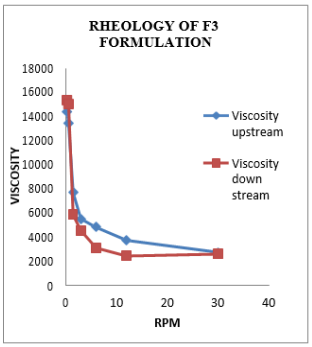
RPM	Viscosity upstream	Viscosity down stream
0.3	14397	15197
0.6	9998	13798
1.5	7198	6358
3	5519	4559
6	4319	3200
12	3439	2449
30	2579	2579
60	EE	EE



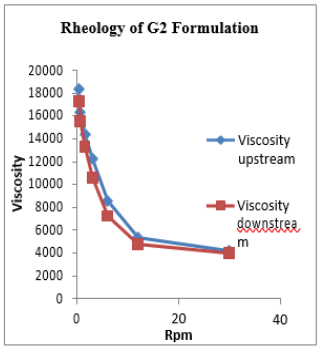
RPM	Viscosity upstream	Viscosity down stream
0.3	18279	17298
0.6	16397	15498
1.5	14124	13298
3	12266	10598
6	8998	7598
12	5379	4779
30	3950	3899
60	EE	EE



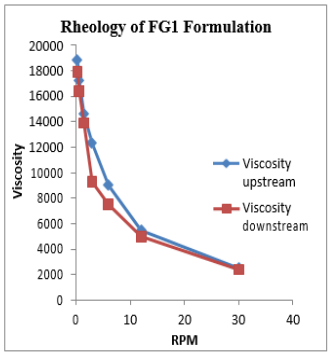
RPM	Viscosity upstream	Viscosity down stream
0.3	14397	15297
0.6	13455	14998
1.5	7698	5858
3	5519	4559
6	4819	3112
12	3739	2449
30	2750	2599
60	EE	EE



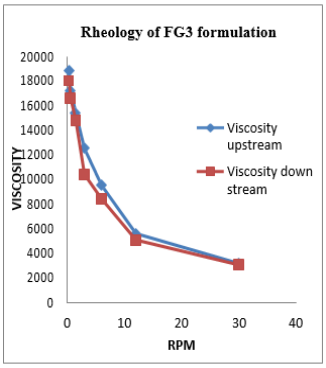
RPM	Viscosity upstream	Viscosity down stream
0.3	18297	17297
0.6	16259	15498
1.5	14352	13298
3	12212	10598
6	8568	7243
12	5348	4779
30	4189	3998
60	EE	EE



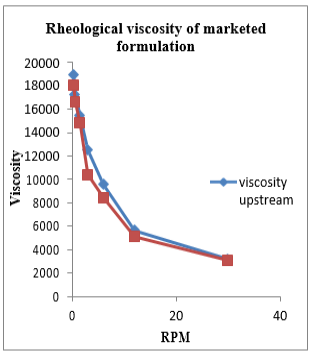
RPM	Viscosity upstream	Viscosity down stream
0.3	18799	17879
0.6	17198	16397
1.5	14589	13892
3	12298	9257
6	8998	7498
12	5458	4989
30	2487	2389
60	EE	EE



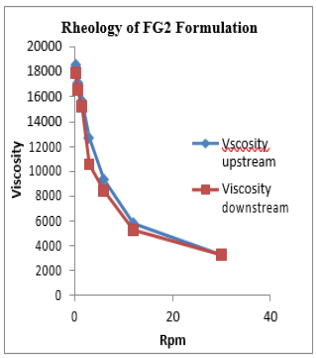
RPM	Viscosity upstream	Viscosity down stream
0.3	18905	18051
0.6	17254	16623
1.5	15455	14854
3	12541	10439
6	9565	8454
12	5645	5123
30	3215	3100
60	EE	EE



RPM	Viscosity upstream	Viscosity down stream
0.3	18989	18124
0.6	16998	16118
1.5	14778	14189
3	11998	10998
6	9768	8989
12	5852	5278
30	3858	3474
60	EE	EE



RPM	Viscosity upstream	Viscosity down stream
0.3	18579	17912
0.6	17117	16576
1.5	15697	15254
3	12697	10559
6	9335	8454
12	5797	5289
30	3257	3278
60	EE	EE



RPM	Viscosity upstream	Viscosity down stream
0.3	18791	17987
0.6	17223	16597
1.5	15297	14214
3	12546	10589
6	9987	8398
12	5059	4276
30	3547	3212

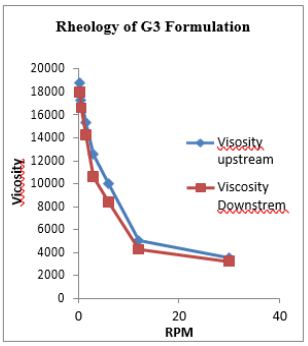


Table 4 Viscosity and Rheograms of optimized formulation F1,F2,F3,G1,G2,G3,FG1,FG2,FG3,Marketed one

4. ANTI-INFLAMMATORY TESTING

Rats' hind paws were subplantarly injected with 0.1 ml (1% W/V) of carrageenan, causing edema, which was then measured using a plethysmometer. Each animal's inflamed paw received the gel formulation as soon as the carrageenan injection was completed. At one, two, and three hours following the gel formulation's application, the edema was measured. For comparisons, the same procedure was used for the Ayurvedic and commercially available diclofenac formulations.

• In vivo paw edema model: Carrageenan injections in the subplantar paw of Wistar albino rats were followed by topical gel application (F3, G3, FG3, and a commercial reference).

• Paw volumes were recorded one, two, and three hours after injection.

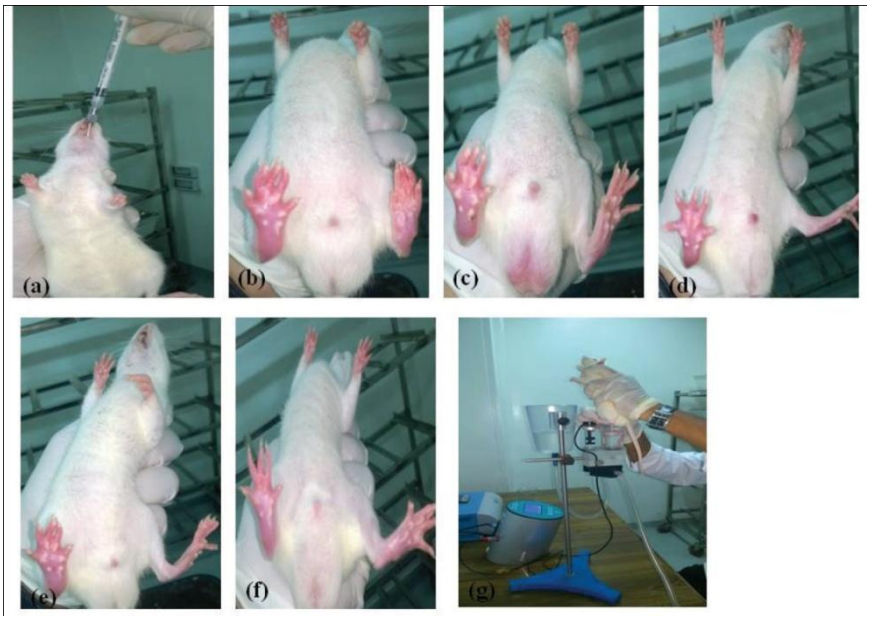


Fig2-Image of Inflammation given to Rats

Group	**Treatment**	**Paw Volume**		
		1hr	**2hr**	**3hr**
1	Control	0.357±0.0239	0.505±0.0239	0.61±0.0238
2	Standard	0.29±0.0302	0.17±0.0108	0.102±0.0062
3	Fenugreek (F3)	0.3±0.0362*	0.13±0.0141*	0.145±0.0301*
4	Garlic (G3)	0.21±0.0264*	0.185±0.0370*	0.122±0.0400*
5	Fenugreek &Garlic (FG3)	0.232±0.0137**	0.11±0.0208**	0.09±0.0147**

Values are expressed as mean ± SEM, *indicate (P < 0.05) vs control; ** indicate (p<0.01) vs control

Table-5 Anti-inflammatory Activity of Optimized formulations

Treatment	**% Edema inhibition**		
	1hr	**2hr**	**3hr**
Standard	18.76	66.33	83.27
Fenugreek (F3)	15.96	74.25	76.22
Garlic(G3)	41.17	63.36	80
Fenugreek &Garlic (FG3)	35.01	78.21	85.24

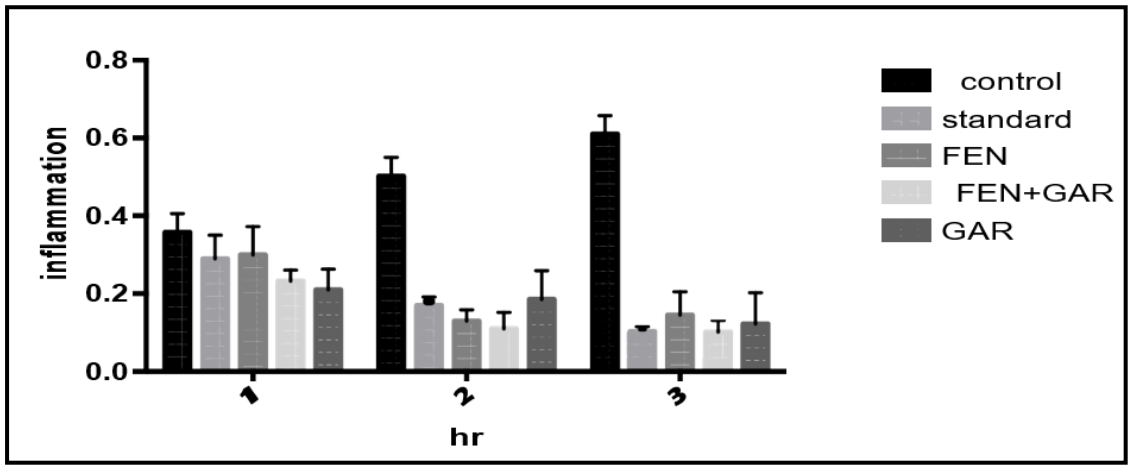
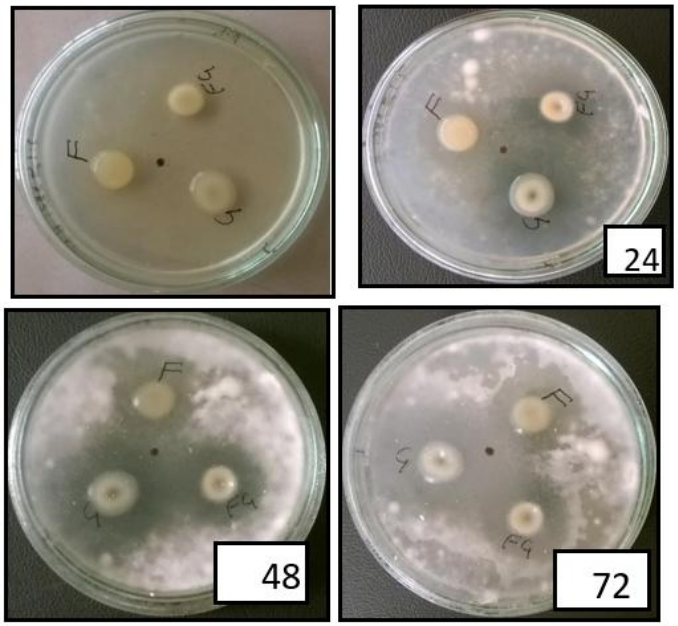


Table 6 & 7-Percentage of Edema Inhibition of Anti-Inflammatory Activity and Anti-Inflammatory activity of Optimized

5. ANTI-FUNGAL ACTIVITY OF OPTIMIZED FORMULATION

Three gels F3, G3 and FG3 were used to check antifungal activity and to determine its minimum inhibitory concentrations. On agar well media with TM fungal strain, showed different zone of inhibition against all three formulations. These formulations have an ability to inhibit growth of fungal strain at varying degrees. From (table34 and figure

32) it is observed that zone of inhibition obtained by G3 formulation was significantly higher than F3 and FG3 formulations may be due to presence of flavonoids in greater extent in garlic extract than fenugreek. Also indicated in previous reports that garlic contains flavonoid allicin which contributes strongly for antifungal activity (Kathi J.,et.al.,(2000)).



eFig 3Agar well media with Trichophyton mentagrophytes (TM) strains after 24,48 and 72 hr of treatment with formulations.

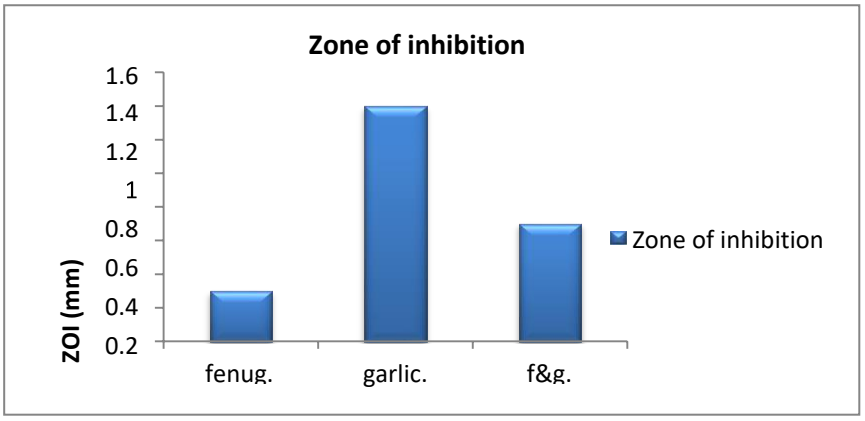


Fig 4 ZOI of formulation against TM Strain

Stability study:

In accordance with ICH guidelines, the stability study was conducted. The gel was tested for appearance, pH, and spreadability after being filled in collapsible tubes and kept for three months at various temperatures and humidity levels, specifically 25±2ºc / 60±5% RH, 30±2ºc / 65±5% RH, and 40±2ºc / 75±5% RH. (Arvind Negi and others, 2012).

The preparations were stable under typical storage conditions, according to the stability study results shown in table 2 above. However, the appearance, pH, spreadability, and extrudability all showed only slight changes from their initial values at higher temperatures and humidity levels.

Formulation	Appearance	pH	Spreadability	Extrudability
F1	Light Yellow (Transparent)	6.6	20	18.24
F2	Light Yellow (Transparent)	6.4	27	19.24
F3	Light Yellow (Transparent)	6.7	32	20.39
G	Light Yellow (Transparent)	6.5	21	18.30
G2	Light Yellow (Transparent)	6.6	28	19.20
G3	Light Yellow (Transparent)	6.7	31	18.89
FG1	Light Yellow (Transparent)	6.4	24	19.40
FG2	Light Yellow (Transparent)	6.6	27	19.11
FG3	Light Yellow (Transparent)	6.8	31	19.69
Marketed Gel	White	6.9	33	20

Table 8-Stability studies of optimized formulation

6. DISCUSSION

The combined topical gel FG3 demonstrated markedly enhanced anti-inflammatory and antifungal activities, attributed to the synergistic phytochemical profiles of *Allium sativum* and *Trigonella foenum-graecum*. The abundant flavonoid and saponin content contribute to radical scavenging and membrane stabilization, while diosgenin offers potent anti-edematous

action comparable to standard anti-inflammatory agents. Notably, the phenolic concentration in fenugreek and higher flavonoid content in garlic complement each other, resulting in maximum biological benefit in FG3 compared to single-extract gels.

The antifungal activity of garlic (G3) aligns with literature reporting allicin's potent action against dermatophytes, while the broad-spectrum efficacy of FG3 suggests the extracts' utility against mixed-pathogen dermatoses

Optimal physicochemical, rheological, and pH attributes favor patient acceptability, enhanced user compliance, and maximal skin bioavailability. Accelerated stability and skin safety findings further bolster product viability for clinical development. As such, dual-extract botanical gels are substantiated as innovative platforms for integrated topical therapy.

7. CONCLUSION

Optimized dual-extract gels containing *Allium sativum* and *Trigonella foenum-graecum* (FG3) offer superior anti-inflammatory and antifungal efficacy, excellent stability, and high skin compatibility. These findings advocate for their continued development toward safe, effective interventions for cutaneous inflammatory and infectious disorders.

8. ACKNOWLEDGEMENT

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Conflict of Interest

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

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