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Research Paper

Development and Evaluation of Polyherbal Gel for Anti-Inflammatory Activity in Superficial Burns

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ABSTRACT

Plant stem cell extracts remain largely underexplored for treating superficial burns. The objective of this study was to formulate and evaluate polyherbal gel for anti-inflammatory activity in superficial burns. The plant stem extracts Centella Reversa CO2, Turmeria Zen and excipients were subjected to preformulation studies. The physicochemical characteristics, the λmax and calibration curve of extracts and drug-excipient interactions were studied. F1- F5 was prepared with different combinations of plant stem cell extracts and evaluated in comparison with 0.5% w/w cetrimide-containing marketed product. The formulations were evaluated for pH, viscosity, spreadability and extrudability etc. and exhibited satisfactory results. The optimized formulation F3 showed about 86% inhibition at 100 µg/mL in HaCaT cells and satisfactory anti-inflammatory activity in RAW 264.7 cell lines, highlighting its strong therapeutic potential and synergistic effects. The short term accelerated stability studies showed no changes in the formulations at the end of one month

INTRODUCTION

The skin is the largest human organ, covering an average surface area of about 1.8 m² in an adult. Structurally, the skin is composed of three major layers- epidermis, dermis and hypodermis. The outermost epidermis provides a waterproof barrier and contributes to skin tone. The middle layer of dermis is rich in collagen, elastin, glands, and vasculature, while the hypodermis provides necessary insulation and cushioning (1). The skin acts as the first line of defense against physical, chemical, and microbial challenges (2). Superficial burns are one of the most common types of skin injuries. It refers to the physiological condition which involves injury to the skin and underlying tissues (3). It can be caused by external stimuli such as heat, radiation, chemical reagents,

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friction or electricity. The severity of the burn is primarily dependent on the nature of the causation stimuli and the depth of injury caused to the skin and underlying tissues (4).

1.1 Need for Study

Conventional treatments for superficial burns, such as silver sulfadiazine, antibiotic ointments and corticosteroids are frequently applied, without considering comorbidities, skin type or burn site, which can lead to variable absorption and negligent use. Among pharmacognostical approaches, *aloe vera*, turmeric and honey have been investigated. Generally, plant stem cell extracts are rich in bioactive compounds, antioxidants and growth factors and have the potential to promote anti- inflammatory activity. Plant stem cell extracts remain largely underexplored for treating superficial burns (5). Thus, plant stem cell extracts should be explored for developing more effective solutions against superficial burns. The aim of the study is to formulate and evaluate polyherbal gel for anti-inflammatory activity in superficial burns using plant stem cell extracts *Centella Reversa* C02 and *Turmeria Zen.*

1.2 Emerging Role of Plant Stem Cell Extracts

Plant stem cell culture is an emerging pharmacognostical approach used for sustainable production of bioactive compounds (6). Typically, plant parts such as meristems, callus tissue, leaf explants, root tips and flower buds are cultured as they contain undifferentiated stem cells which are capable of regeneration (7). Major plant stem cell culturing techniques include callus culture, suspension culture, hairy root culture and organogenesis (8). Plant stem cell extracts are bioactive- rich substances obtained by lysis or processing plant stem cells, rather than using the living cells directly. These extracts are derived from primitive, undifferentiated plant stem cells and contain antioxidants, peptides, phytohormones and other beneficial molecules (9).

1.3 *Centella Reversa C02 and Turmeria Zen*

Centella Reversa C02 is a phytopeptidic fraction of stem cells derived from *C. asiatica*. It contains a proprietary blend of *C. asiatica* stem cell extract, glycerine, sodium benzoate and calcium gluconate. The production of *Centella Reversa* C02 follows a systematic biotechnological process starting with the initiation of cell culture from *C. asiatica* leaf explant. A growth boosting step is introduced, leading to the development of a high-density cell culture. The dense culture is then subjected to filtration, ensuring the removal of unwanted materials. The next stage involves the collection of conditioned media, which contains the desired bioactive molecules secreted during the culture process. This conditioned media is processed into a usable form suitable for cosmetic or therapeutic applications (10).

Turmeria Zen includes plasma rich in cell factors, derived from stem cells of *C. longa*. It is a proprietary blend of *C. longa* callus lysate, glycerine and citric acid. When stimulated with plant stress hormones and osmotic stress, turmeric stem cells generate a metabolome (PRCF) enriched with stress-response factors such as diarylheptanoids, phytosterols, terpenes, saponins, osmolytes, sugars, lipids, and low molecular weight proteins. With high levels of polysaccharides (10,000 ppm, including glucose, fructose, myo-inositol, and sucrose), *Turmeria Zen* enhances water retention, provide osmoprotection and helps in skin repair (11).

2. Methodology

2.1 Procurement and Authentication of Ingredients

The plant stem cell extracts chosen for the study, *Centella Reversa* C02 and *Turmeria Zen*, were obtained as gift samples from Chemical Brothers, Hyderabad, India. Euxyl K-903, a preservative, was procured from Nice Company, Kerala. Commercially available anti-inflammatory product was procured from online resource.

1A 1B 1C 1D 1E

Fig.1 Photographs of the pharmaceutical ingredients employed 1A- *Turmeria Zen*, 1B- *Centella Reversa C02,* 1C- Euxyl K-903, 1D- Menthol, 1E- Marketed Product

2.2 Preformulation Studies

2.2.1 Appearance and Odour

The appearance of the ingredients was checked by visual inspection. The odour of the ingredients was inspected.

2.2.2 Solubility

The solubility of the pharmaceutical ingredients was determined using distilled water and ethanol (12).

2.2.3 pH

The digital pH meter was calibrated by standard buffers. The pH electrode was thoroughly rinsed with distilled water and wiped dry. The pH electrode was dipped in sample and reading was noted (13).

2.2.4 Density and Specific Gravity (14)

Pycnometer was used to find out the density and specific gravity of the sample. A clean dry glass pycnometer was taken and the weight was noted. The bottle was filled with the sample to overflowing and reweighed (47). The weights were noted and parameters were calculated as follows.

Weight of the specific gravity bottle = W1 g

Weight of the specific gravity bottle + 10 mL of distilled water = W2 g Weight of the specific gravity bottle + 10 mL of sample = W3 g

Density of sample (in g/ mL) = *(Weight of sample X Density of water) / Weight of sample*

Specific Gravity= *Density of sample/ 0.9963*

2.2.5 Determination of λ_{max} (15)

The stock solution A was prepared by dissolving 1 mL of plant stem cells in 10 mL of distilled water (Concentration= 100µL of plant stem cells/ mL of solution). The serial dilutions were prepared by taking aliquots of 0.1, 0.2, 0.3, 0.4, and 0.5 mL of stem cell extract into separate volumetric flasks and making up the volume with distilled water upto 10mL. Each dilution was scanned between 200–800 nm. The absorbance was recorded to identify λ_{max} corresponding to maximum absorbance. The absorbance was recorded. The calibration curve was drawn.

2.2.6 Drug- Excipient Interaction

The plant stem cells CR C02, TZ and a physical mixture was subjected to FTIR studies to identify the molecular composition of the sample and checked for drug-excipient interaction (16).

2.3 Formulation Studies

Polyherbal gel formulations for superficial burns were developed using plant stem cells extracts, Carbopol 934, menthol and Euxyl K-903. The prepared formulations (F1–F5) are summarized in **Table 1.**

Table 1: Formulation Chart (F1 to F5)

		Formulation Code				
S. No.	**Ingredients (Category)**	**F1**	**F2**	**F3**	**F4**	**F5**
1.	*Centella Reversa* C02 (API, Anti-inflammatory agent)	0.2 mL	0.15 mL	0.1 mL	0.05 mL	-
2.	*Turmeria Zen* (API, Anti-inflammatory agent)	-	0.05 mL	0.1 mL	0.15 mL	0.2 mL
3.	Menthol (Cooling agent)	0.1 g	0.1g	0.1g	0.1g	0.1g
4.	Carbopol 934 (Gelling Agent)	0.11g	0.12 g	0.13 g	0.14 g	0.15 g
5.	Triethanolamine (pH modifier)	0.45 mL	0.45 mL	0.45 mL	0.45 mL	0.45 mL
6.	Propylene glycol (Viscosity Modifier)	*q.s.*	*q.s.*	*q.s.*	*q.s.*	*q.s.*
7.	Euxyl K-903 (Preservative)	0.2 mL	0.2 mL	0.2 mL	0.2 mL	0.2 mL
8.	Distilled water (Vehicle)	20 mL	20 mL	20 mL	20 mL	20 mL

2.3.1 Formulation Procedure (17)

The formulations, given in **Table 1,** were prepared by simple agitation using magnetic stirrer. Duplicate sets of the formulations were prepared such that one set was used for evaluation and the other set was used for stability studies. Accurate quantity of DM water, plant stem cell extracts and Carbopol 934 was measured using measuring cylinder and digital weighing balance respectively and transferred to a dry, clean glass beaker. Carbopol 934 was allowed to disperse uniformly in the active ingredients using a magnetic stirrer with a magnetic bead and allowed to swell for atleast 15- 20 minutes. The aqueous phase containing the active ingredients are transferred slowly. 0.1 g of menthol was dissolved in 0.2 mL of Euxyl K-903 in a separate beaker and dispersed uniformly in the aqueous phase. 0.45 mL of TEA was added dropwise with continous stirring until the gel formation was achieved. Propylene glycol was added *q.s* to prevent moisture loss and achieve optimal viscosity. The gel was transferred into clean, wide mouth plastic container with screwcap and labeled.

2.4 Evaluation Studies

The evaluation of the polyherbal gel formulations (F1- F5) was performed. For comparison, the marketed product with 0.5% w/w cetrimide was also evaluated.

2.4.1 Organoleptic Properties

The appearance and odour were determined as explained in **Sec 2.2.1** respectively.

2.4.2 Gel Yield

The gel formation yield was evaluated by comparing the theoretical total weight of ingredients with the practical weight of gel obtained. Theoretical weight was calculated by summing the masses of all ingredients, while the prepared gel was carefully weighed (18).

Percentage Yield (%) = (Practical Yield (g) X 100) / Theoretical Yield (g)

2.4.3 pH

The pH of the formulations and marketed product was determined as explained in **Sec 2.2.3** respectively.

2.4.4 Viscosity

The viscosity of the gel formulations was determined using a Brookfield Viscometer (Model DV-III); equipped with spindle no. 64. A 50 mL sample of each formulation was placed in the sample holder, and measurements were taken at 200 rpm for 30 seconds at 25 ± 1 °C. The average viscosity was recorded in centipoise (cP) (19).

2.4.5 Spreadability

The spreadability of the gel formulations was determined using the two-glass-slide method. About 1 g of gel was placed between two glass slides. A weight of 5 g was placed on the upper slide at 0 seconds, followed by an additional 5 g at 15 seconds. At 30 seconds, the spreading diameter of the gel was measured. The procedure was repeated three times for each formulation, and the average value of spreadability and surface area of the gel spread was reported (20).

Spreadability = ***Mass added (g) X Diameter of the gel circle after spreading (cm)***
Time taken (s)

Surface area of the gel spread = ***3.14 X (Diameter of the gel circle/ 2)^2***

2.4.6 Extrudability

The extrudability was evaluated by filling the prepared gel into a collapsible plastic tube. The tube was pressed between fingers, and the amount of gel extruded in 10 seconds was collected. The extrudability was classified as easily, moderately or poorly extrudable (21).

2.4.7 In- Vitro Anti-Inflammatory Activity

2.4.7.1 Using HaCaT Cell Line (22)

The anti-inflammatory activity of the optimized formulation was compared with the marketed preparation using HaCaT keratinocyte cell lines. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, maintained under standard culture conditions (37 °C, 5% CO_2, 95% relative humidity). For experimentation, HaCaT cells were seeded in 24-well plates at a density of 2×10^5 cells/well and allowed to adhere for 24 hours. Inflammatory response was induced by treatment with lipopolysaccharide (LPS, 1 µg/mL) for 6 hours. After induction, the medium was replaced with fresh DMEM containing different concentrations (25, 50, 75, and 100 µg/mL) of the optimized formulation and the marketed sample. Untreated cells were used as negative controls, whereas LPS-stimulated cells without further treatment served as positive controls. The percentage inhibition of inflammatory markers relative to LPS-treated controls was calculated to establish the comparative anti-inflammatory efficacy of the optimized formulation and marketed preparation.

2.4.7.2 Using RAW 264.7 Cell Line (23)

RAW 264.7 macrophage cells were cultured in DMEM supplemented with 10% FBS and 1% antibiotics at 37 °C with 5% CO_2. For cytotoxicity screening, cells were seeded at 2.5×10^4 cells/well and treated with different concentrations (25, 50, 75, and 100 µg/mL) of the optimized formulation and the marketed sample. Cell viability was assessed using the MTT assay, with absorbance measured at 520 nm. Anti-inflammatory activity was evaluated by measuring nitric oxide (NO) release following stimulation with 1 µg/mL LPS. Nitrite levels in the culture supernatant were quantified using Griess reagent at 540 nm, with sodium nitrite as the standard.

2.5 Short- Term Accelerated Stability Studies

The preliminary stability studies were conducted for formulations F1– F5 at 40 ± 2°C, 75 ± 5% RH, for one month. The gel samples were inspected for changes in appearance, odour and pH (24).

3. RESULTS AND DISCUSSION

3.1 Preformulation Studies

3.1.1 Appearance and Odour

Table 2: Appearance and Odour of Procured Ingredients

S. No.	Ingredient	Appearance	Odour
1.	*Centella Reversa* C02	Off white	Characteristic
2.	*Turmeria Zen*	Yellow	Characteristic
3.	Carbopol 934	White	Characteristic
4.	Triethanolamine	Colorless	Ammoniacal
5.	Propylene glycol	Colorless	Sweet
6.	Euxyl K-903	Golden yellow	Slightly aromatic
7.	Menthol	Colorless	Characteristic minty

3.1.2 Solubility

Table 3: Solubility of the Procured Ingredients

S. No.	Ingredient	Quantity	Solubility in water	Solubility in ethanol
1.	*Centella Reversa* C02	0.1 mL	Soluble	Soluble
2.	*Turmeria Zen*	0.1 mL	Soluble	Soluble
3.	Carbopol 934	0.1 g	Soluble	Insoluble
4.	Menthol	0.1 g	Insoluble	Soluble
5.	Euxyl K-903	0.1 mL	Uniformly dispersed	Soluble

CR C02 and TZ were soluble in water and ethanol, indicating good compatibility for aqueous-based gel formulations. Carbopol 934 was soluble only in water while menthol displayed water insolubility but good ethanol solubility. The solubility data confirms the feasibility of incorporating all ingredients into a gel base.

3.1.3 pH

Table 4: pH of the Procured Ingredients

S. No.	Ingredient	pH
1.	*Centella Reversa* C02	4.0
2.	*Turmeria Zen*	3.2
3.	Carbopol 934	2.4
4.	Menthol	5.5
5.	Euxyl K-903	5.7
6.	Triethanolamine	10.2

CR C02 and TZ showed slightly acidic pH values, while excipients like TEA exhibited alkaline pH (10.2). Carbopol 934 was highly acidic. This wide pH range suggests that neutralization with TEA is essential for gel formation and stabilization.

3.1.4 Density and Specific Gravity

Table 5: Density and Specific Gravity of Extracts

S. No.	Ingredient	Density (g/ mL)	Specific Gravity
1.	*Centella Reversa* C02	1.168	1.173
2.	*Turmeria Zen*	1.187	1.191

Both plant stem cell extracts displayed high densities (>1.16 g/mL), slightly above water.

3.1.5 Determination of λ_{max}

Table 6: Determination of λ_{max} of *Turmeria Zen*

S. No.	Volume taken (mL)	Concentration (µL/mL)	Maximum wavelength (λ_{max})
1.	0.6	6	228 nm

Table 7: Calibration Data of *Turmeria Zen*

S. No.	Volume taken (mL)	Concentration (µL/mL)	Absorbance
1	0.1	1	0.2889
2	0.2	2	0.4141
3	0.3	3	0.5495
4	0.4	4	0.6925
5	0.5	5	0.8156
6	0.6	6	0.9025

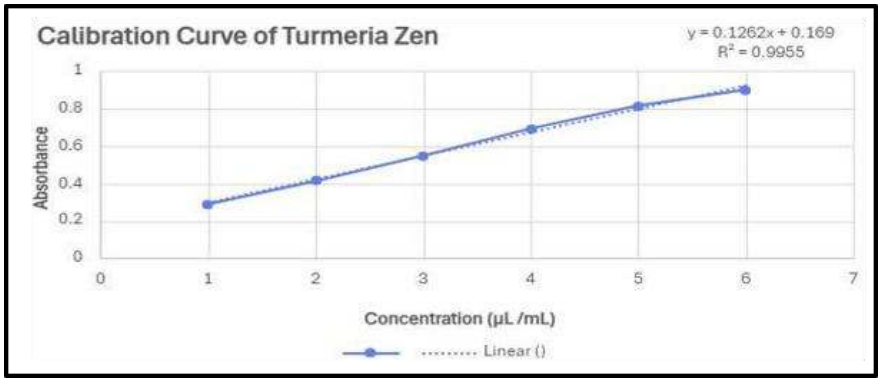

Fig. 2: Calibration Data Curve of *Turmeria Zen*

Table 8: Determination of λ_{max} of *Centella Reversa* C02

S. No.	Volume taken (mL)	Concentration (µL/ mL)	Maximum wavelength (λ_{max})
1.	0.5	5	232 nm

Table 9: Calibration Data of *Centella Reversa* C02

S. No.	Volume taken (mL)	Concentration (µL/mL)	Absorbance
1.	0.1	1	0.5728
2.	0.2	2	0.6745
3.	0.3	3	0.8123
4.	0.4	4	0.9622
5.	0.5	5	1.0775

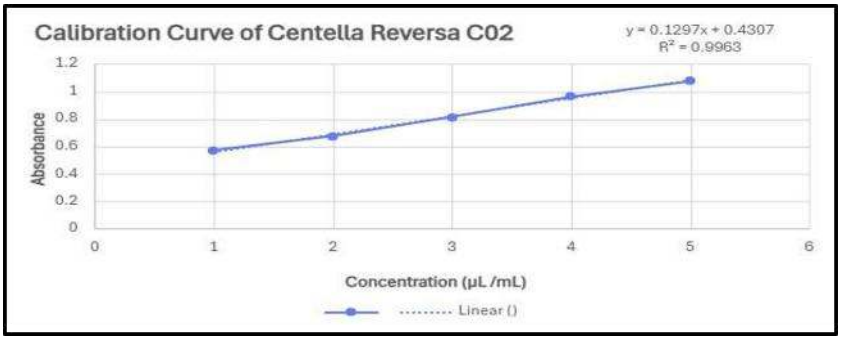

Fig. 3: Calibration Curve of *Centella Reversa* CO2

The λ_{max} of TZ was found to be 228 nm, while CR C02 exhibited a λ_{max} at 232 nm. Calibration curves of both extracts showed good linearity between concentration and absorbance, confirming their compliance with Beer–Lambert's law.

3.1.6 Drug-Excipient Interaction

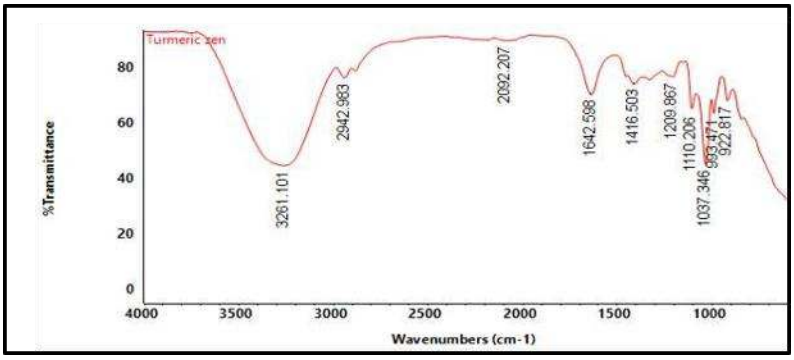

Fig. 4: FTIR Spectrum of *Turmeria Zen*

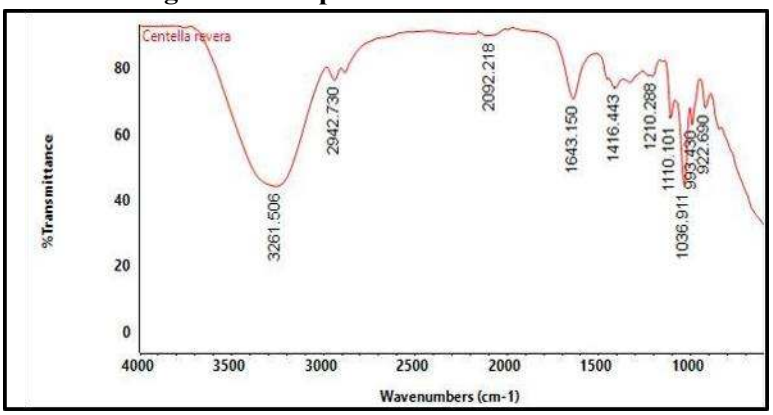

Fig. 5: FTIR Spectrum of *Centella Reversa* C02

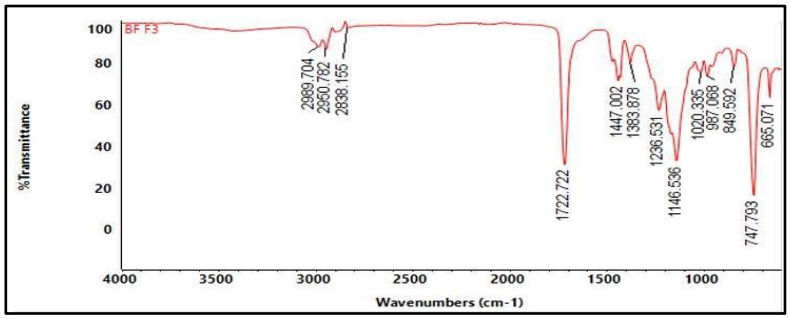

Fig. 6: FTIR Spectrum of Physical Mixture

3.2 Formulation Studies

All the formulations F1-F5 as given in **Table 1** could be successfully prepared by simple agitation and were found to be homogenous as shown in **Fig. 7.**

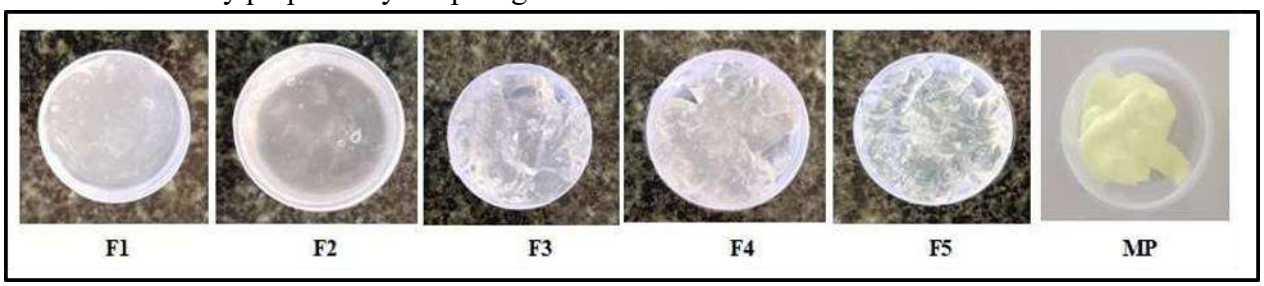

Fig. 7: Formulations F1 – F5 and MP

3.3 Evaluation Studies

3.3.1 Appearance and Odour

Table 10: Appearance and Odour of the F1-F5 and MP

S. No.	Code	Appearance	Odour
1.	F1	Colorless	Odourless
2.	F2		
3.	F3		
4.	F4		
5.	F5		
6.	MP	Pale yellow	Characteristic

3.3.2 Gel Yield

Table 11: Percentage Gel Yield

S. No.	Code	Theoretical Yield (g)	Practical Yield (g)	Percentage Yield (%)
1.	F1	21.21	20.91	98.6
2.	F2	21.22	20.88	98.4
3.	F3	21.35	21.09	98.8
4.	F4	21.44	21.24	99.1
5.	F5	21.45	21.19	98.8

All formulations showed high yield (>98%), with F4 giving the maximum yield (99.1%). The close agreement between theoretical and practical yields indicates minimal processing losses and good reproducibility of the method.

3.3.3 pH

Table 12: pH of F1-F5 and MP

S. No.	Code	pH
1.	F1	8.06
2.	F2	7.48
3.	F3	7.36
4.	F4	7.25
5.	F5	7.63
6.	MP	5.92

Formulations F1–F5 exhibited pH values between 7.25 and 8.06, which are within the skin-compatible range (6–8). In contrast, the marketed preparation showed a slightly lower pH (5.92), indicating more acidic nature. The optimized formulation‘s near-neutral pH ensures reduced irritation potential.

3.3.4 Viscosity

Table 13: Viscosity of F1-F5 and MP

S. No.	Code	Viscosity (cP)
1.	F1	35856
2.	F2	36692
3.	F3	37564
4.	F4	38350
5.	F5	40344
6.	MP	38214

Viscosity increased progressively from F1 (35856 cP) to F5 (40344 cP), suggesting that Carbopol and TEA ratios directly influenced gel strength. F5 exhibited the highest viscosity, indicating more consistency, whereas F1 was more spreadable. The marketed preparation had intermediate viscosity (38214 cP), showing comparability with the developed formulations.

3.3.5 Spreadability

Table 14: Spreadability of F1-F5 and MP

S. No.	Code	Spreadability (g.cm/ sec)	Surface Area (cm^2)
1.	F1	1.06	6.594
2.	F2	1.6	11.938
3.	F3	1.03	4.615
4.	F4	1.03	2.833
5.	F5	0.87	3.661
6.	MP	0.8	1.539

F2 showed maximum spreadability (1.6 g·cm/sec), while F5 showed the least (0.87 g·cm/sec). This inverse relationship between viscosity and spreadability is expected: more viscous gels spread less easily.

3.3.6 Extrudability

Table 15: Extrudability of F1-F5 and MP

S. No.	Code	Extrudability
1.	F1	Easy
2.	F2	Easy
3.	F3	Easy
4.	F4	Moderate
5.	F5	Moderate
6.	MP	Moderate

F1–F3 were easily extrudable, while F4, F5, and MP showed moderate extrudability due to their higher viscosity. Easy extrusion is desirable for patient compliance, indicating that F1–F3 are favorable in this aspect.

3.3.7 *In-Vitro* Anti-Inflammatory Activity

Table 16: Percentage Inhibition for *In-Vitro* Anti- Inflammatory Activity

		HaCaT Cell Line		RAW 264.7 Cell Line	
S. No.	**Concentration (µg/mL)**	**% Inhibition by F3**	**% Inhibition by MP**	**% Inhibition by F3**	**% Inhibition by MP**
1.	25	19.23	26.92	18.33	13.33
2.	50	30.76	38.46	23.33	31.66
3.	75	53.84	57.69	43.33	41.66
4.	100	86.45	84.61	48.33	56.66

On HaCaT and RAW 264.7 cell lines, F3 demonstrated potent inhibition, comparable to or exceeding the marketed preparation at higher concentrations. Notably, F3 achieved ~86% inhibition at 100µg/mL in HaCaT cells, highlighting its strong therapeutic potential. This confirms the synergistic effect of CR C02 and TZ.

3.4 Short- Term Accelerated Stability Studies

Table 17: Stability Study Parameters of F1- F5

S. No.	Code	Appearance	Odour	pH
1.	F1	Colorless	Odourless	8.02
2.	F2			7.46
3.	F3			7.27
4.	F4			7.21
5.	F5			7.04

All formulations remained stable in terms of appearance, odour and pH during short-term evaluation. Minor changes in pH (within acceptable range) were observed, but no significant degradation or phase separation occurred. This confirms physical and chemical stability of the optimized gel.

CONCLUSION

The findings suggest that the development of polyherbal gels using plant stem cell extracts enriched with plant stem cell extracts can provide a better alternative for managing inflammatory conditions as in burns. Such gels may offer safer, residue-free solutions compared to conventional anti-inflammatory drugs, addressing concerns of adverse effects, resistance, long-term tissue impact while promoting sustainable therapeutic approaches.

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Conflict of Interest: None

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