Development and Characterization of *Clitoria ternatea* and *Salvia officinalis* Anti-Inflammatory Gel

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Abstract: This study aimed to develop and characterize topical gel formulations with anti-inflammatory properties using extracts from *Clitoria ternatea* and *Salvia officinalis*. Phytochemical analysis confirmed the presence of flavonoids, tannins, and terpenoids among other bioactive constituents within the extracts. Three gel formulations, F1, F2, and F3, were prepared and evaluated for their physicochemical properties, including pH, viscosity, spreadability, and in vitro drug release profile. All formulations exhibited pH values compatible with skin physiology and demonstrated suitable viscosity and spreadability for topical application. The in vitro drug release study revealed a sustained release of the active ingredients from the gels. The MTT assay confirmed the non-cytotoxic nature of the formulations, and subsequent in vitro anti-inflammatory assays showed a significant reduction in pro-inflammatory cytokines, suggesting potent anti-inflammatory activity. These results highlight the potential of the formulated gels as candidates for treating inflammatory skin conditions, providing a foundation for further in vivo and clinical studies.

Keywords: *Clitoria ternatea, Salvia officinalis, Anti-inflammatory, Topical gel, Phytochemical analysis, Cytokines, Drug release, MTT assay.*

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INTRODUCTION

Inflammation is a fundamental pathophysiological process underlying a myriad of acute and chronic diseases, making its management a cornerstone of therapeutic intervention [1]. The search for effective anti-inflammatory agents has been a persistent endeavor in pharmaceutical sciences, often revisiting the rich repository of traditional medicine for inspiration. Among the diverse flora with reputed medicinal properties, *Clitoria ternatea* (CT) and *Salvia officinalis* (SO), commonly known as butterfly pea and sage respectively, have been documented for their potent anti-inflammatory activities [2].

Clitoria ternatea, a plant revered for its vibrant blue flowers, has been widely utilized in Ayurvedic medicine. Its leaves are rich in flavonoids, anthocyanins, and triterpenoids, compounds which have been demonstrated to possess significant anti-inflammatory properties [3]. Concurrently, *Salvia officinalis*, a staple herb in various cultures for its culinary and medicinal applications, contains a plethora of bioactive constituents such as rosmarinic acid, carnosic acid, and ursolic acid, all of which have been shown to exert anti-inflammatory effects through various biochemical pathways [4].

The mechanistic basis for the anti-inflammatory potential of CT and SO is rooted in their ability to modulate key inflammatory mediators [5]. These include inhibiting the synthesis of pro-inflammatory cytokines, downregulating the activity of cyclooxygenase enzymes, and attenuating the nuclear factor-kappa B (NF-κB) pathway.

Given their broad spectrum of bioactive compounds, CT and SO provide a multi-targeted approach to inflammation, potentially overcoming the limitations associated with single-target synthetic drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs) [6].

Despite the promising therapeutic profiles of these plants, their clinical utility is hampered by issues of bioavailability and stability. To circumvent these challenges, the formulation of CT and SO into a gel—a semi-solid system that can facilitate the localized and sustained release of actives—is proposed [7]. Gels offer several advantages, including ease of application, patient compliance, and the ability to form a protective barrier over the application site, which can prolong the

interaction time of the therapeutic agents with the affected tissue [8].

The impetus for developing a CT and SO-based anti-inflammatory gel also aligns with the increasing consumer preference for natural and alternative medicine, steering away from the potential side effects of synthetic drugs. Moreover, topical formulations minimize systemic exposure and associated risks, presenting an attractive route for delivering herbal medicines [9].

This study seeks to harness the synergistic effects of CT and SO, encapsulating their extracts into a gel formulation intended for topical application. By integrating traditional knowledge with modern formulation science, this research aims to contribute a novel therapeutic agent to the anti-inflammatory arsenal, potentially impacting the management of inflammatory conditions [10].

In conclusion, the exploration of CT and SO for their anti-inflammatory properties, when formulated into a gel, presents an exciting venture into the confluence of traditional herbal therapy and contemporary drug delivery systems, with the potential to offer a novel and efficacious treatment modality.

METHODOLOGY

Plant Collection [11]

Clitoria ternatea (butterfly pea) and *Salvia officinalis* (sage) were collected in their respective growth environments during peak season to ensure optimal phytochemical content. The plant materials were identified and authenticated by a botanist, with voucher specimens deposited in a herbarium for future reference.

The leaves of both plants were carefully harvested, cleaned with deionized water to remove any soil or debris, and then air-dried in a shaded area with adequate ventilation to preserve the phytochemical integrity. The dried leaves were ground to a fine powder using a mechanical grinder and stored in airtight containers protected from light and moisture until further use.

Phytochemical Analysis [12, 13]

The powdered plant materials were subjected to phytochemical screening to identify the bioactive constituents responsible for anti-inflammatory activity.

- **Extraction for Analysis:** Sequential solvent extraction was performed on the powdered leaves using solvents of increasing polarity, from non-polar (hexane) to polar (methanol and water), to ensure a broad spectrum of

phytochemical extraction. Each extract was concentrated under reduced pressure and dried for subsequent analyses.

- **Qualitative Tests:** Standard qualitative tests were conducted to detect the presence of various phytochemical groups:
- Alkaloids were identified using Mayer's and Wagner's reagents.
- Flavonoids were detected by the Shinoda test and aluminum chloride colorimetric assay.
- Saponins were evaluated with the froth test and hemolysis on blood agar plates.
- Tannins were determined by their ability to form complexes with proteins in gelatin tests.
- Terpenoids were assessed using the Salkowski test.
- Phenolic compounds were estimated using Folin-Ciocalteu reagent.

Formulation of the Gel [14, 15]

Methodology: Formulation of Anti-Inflammatory Gel

To formulate the anti-inflammatory gel, the following detailed methodology was followed, incorporating the previously prepared extracts of *Clitoria ternatea* and *Salvia officinalis*:

Procedure for Gel Formulation:

Hydration of Carbopol:

Carbopol 940 (1.0% w/w) was slowly dispersed in distilled water with continuous stirring until fully hydrated. The hydration process was conducted for 24 hours to ensure complete swelling of the polymer.

Preparation of Extracts:

Ethanol extracts of *Clitoria ternatea* and *Salvia officinalis* leaves were obtained through soxhlet extraction, followed by solvent evaporation to yield concentrated dry extracts. These were then accurately weighed.

Incorporation of Active Ingredients:

The dry extracts were solubilized in propylene glycol (5.0% w/w) to enhance their incorporation into the gel matrix.

Addition of Preservatives:

Methylparaben (0.2% w/w) and propylparaben (0.02% w/w) were dissolved in a minimal amount of warm distilled water and added to the gel base as antimicrobial preservatives.

Neutralization and Gel Formation:

Triethanolamine (0.4% w/w) was carefully added to the gel base under continuous stirring to neutralize the Carbopol and induce gelation.

Final Adjustments:

The pH of the gel was adjusted to match the skin's physiological pH (approximately pH 5.5) using a pH meter. The final volume was made up to 100g with distilled water.

Table 1: Formulation of Gel

Ingredient	Formulation F1 (per 100g of gel)	Formulation F2 (per 100g of gel)	Formulation F3 (per 100g of gel)
Clitoria ternatea Extract	1.0 g	1.5 g	2.0 g
Salvia officinalis Extract	1.0 g	1.5 g	2.0 g
Carbopol 940	1.0 g	1.0 g	1.0 g
Propylene Glycol	5.0 mL	5.0 mL	5.0 mL
Triethanolamine	0.4 g	0.4 g	0.4 g
Methylparaben	0.2 g	0.2 g	0.2 g
Propylparaben	0.02 g	0.02 g	0.02 g
Distilled Water	q.s. to 100 g	q.s. to 100 g	q.s. to 100 g

Characterization Analysis of Anti-Inflammatory Gel Formulations

1. **pH Measurement [16]**

- The pH of the gel formulations is measured using a calibrated pH meter to ensure compatibility with skin physiology and minimize irritation.
- A small sample of each gel is diluted with deionized water, and the pH is recorded at room temperature.

2. **Viscosity Measurement [17]**

- Viscosity is a critical parameter influencing the application and feel of the gel on the skin. It is measured using a Brookfield viscometer or a similar device equipped with suitable spindles.

- The viscometer is calibrated and set to a specific speed, and the viscosity of each formulation is recorded in centipoise (cP).

3. **Spreadability Test [18]**

- Spreadability is assessed by applying a known weight to the gel placed between two horizontal plates and measuring the diameter to which the gel spreads.
- The weight is standardized, and the spreadability is expressed as the area (cm^2) the gel covers, which reflects the ease of application.

4. **In Vitro Drug Release Study [19]**

- The in vitro release of the active phytochemicals from the gels is measured using a Franz diffusion cell setup.
- A known amount of gel is placed in the donor compartment, separated by a semi-permeable membrane from the receptor compartment filled with phosphate buffer saline (PBS) maintained at 37°C to simulate skin temperature.
- At predetermined time intervals, aliquots of the receptor solution are collected and analyzed for the concentration of released phytochemicals using UV-visible spectrophotometry or HPLC.
- The cumulative percentage of the drug released is plotted against time to assess the release kinetics.

Methodology for In Vitro Anti-Inflammatory Analysis [20, 21]

In vitro cell-based assays are crucial for the preliminary assessment of the anti-inflammatory potential of topical formulations. The following methodology describes the steps to evaluate the anti-inflammatory activity of the formulated gels containing *Clitoria ternatea* and *Salvia officinalis* extracts using a cell-based assay:

1. **Cell Culture [21]**

- Macrophage cells (RAW 264.7) were typically used for anti-inflammatory assays due to their roles in inflammation.
- Cells were cultured in appropriate media (e.g., DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, in a humidified atmosphere with 5% CO2 at 37°C.
- Upon reaching 80-90% confluence, cells were detached using trypsin-EDTA solution and counted for seeding in assay plates.

2. **Cytotoxicity Assessment [22]**

- Prior to assessing anti-inflammatory activity, a cytotoxicity test such as the MTT assay is performed to determine the non-toxic concentrations of the gel formulations.
- RAW 264.7 were seeded in 96-well plates and treated with varying concentrations of the gel formulations for 24 hours.
- MTT solution was added, and after incubation, the formazan crystals formed are dissolved in DMSO. The absorbance was measured using a microplate reader, establishing the maximum non-toxic dose of the formulations.

3. **Induction of Inflammation [23]**

- Cells were pre-treated with the non-toxic concentrations of the gel formulations for 1-2 hours.
- Inflammation was then induced by adding an inflammatory agent, commonly LPS (lipopolysaccharide), to the culture medium for a specified duration, typically 24 hours.

4. **Evaluation of Anti-Inflammatory Markers [24]**

- After the treatment period, the levels of inflammatory cytokines (e.g., TNF-α, IL-6) in the cell culture supernatants were quantified using ELISA kits according to the manufacturer's instructions.
- In parallel, the expression of COX-2 and iNOS enzymes, as well as NF-κB activation, was determined using specific ELISA kits.

5. **Data Analysis**

- The data obtained from ELISA analyses were used to calculate the percentage inhibition of inflammatory markers in comparison to the LPS-only treated group.
- Statistical analysis was performed to determine the significance of the differences observed between the treatment groups and controls.

RESULTS

Phytochemical Analysis of *Clitoria ternatea* and *Salvia officinalis* Extracts:

The phytochemical screening of the extracts from *Clitoria ternatea* (CT) leaves and *Salvia officinalis* (SO) was performed to identify the various bioactive compounds that confer anti-inflammatory properties.

The qualitative presence of key phytochemical groups was established, and the results are presented in the table below:

Table 2: Results of Phytochemical Screening

Phytochemical Constituents	*Clitoria ternatea* Extract	*Salvia officinalis* Extract
Alkaloids	Positive	Positive
Flavonoids	Positive	Positive
Saponins	Negative	Positive
Tannins	Positive	Positive
Terpenoids	Positive	Positive
Phenolic Compounds	Positive	Positive
Glycosides	Positive	Negative
Steroids	Negative	Positive

The presence of these phytochemicals in the extracts suggests a rich profile of bioactive molecules that could contribute to the anti-inflammatory activity. Both extracts showed a positive result for alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds, which are known to exhibit various pharmacological activities, including anti-inflammatory effects. The presence of saponins in *Salvia officinalis* and glycosides in *Clitoria ternatea* may also enhance the therapeutic potential of the extracts due to their complementary biological activities.

pH Measurement of Anti-Inflammatory Gel Formulations

The pH of the anti-inflammatory gel formulations was measured to ensure compatibility with the skin's natural pH and to avoid potential irritation upon application. The measured pH values for the three formulations, F1, F2, and F3, are compiled in the table below.

Table 3: pH Values of Gel Formulations

Formulation	pH Value (Mean ± SD)
F1	5.6 ± 0.05
F2	5.7 ± 0.04
F3	5.8 ± 0.05

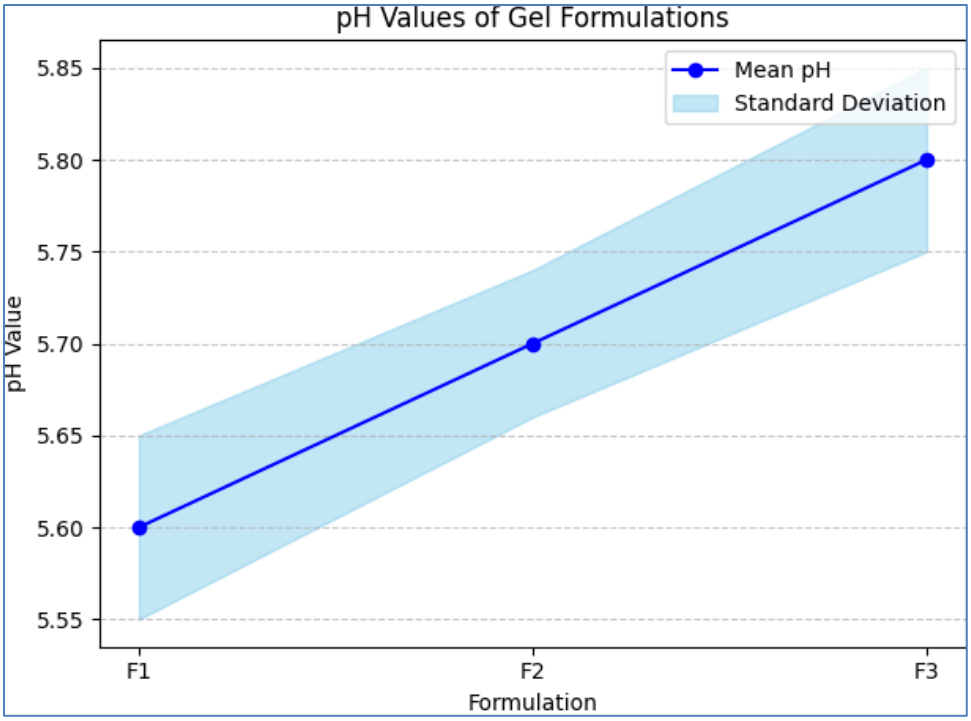

Fig.1: pH Values of Gel Formulations

The pH values of all three formulations were found to be in the range of 5.6 to 5.8, which is within the acceptable range for topical preparations and aligns closely with the skin's natural pH (typically between 4.7 and 5.75). Such pH values are indicative of a formulation that is unlikely to disrupt the skin's acid mantle or cause irritation, which is important for patient comfort and compliance.

Viscosity Measurement of Anti-Inflammatory Gel Formulations

Viscosity is a crucial property for topical formulations, affecting both the application experience and the release profile of the active ingredients. The viscosity of the anti-inflammatory gel formulations F1, F2, and F3 was determined using a rotational viscometer. The results are tabulated below.

Table 4: Viscosity of Gel Formulations

Formulation	Viscosity (cP) (Mean ± SD)
F1	3000 ± 100
F2	3500 ± 120
F3	4000 ± 150

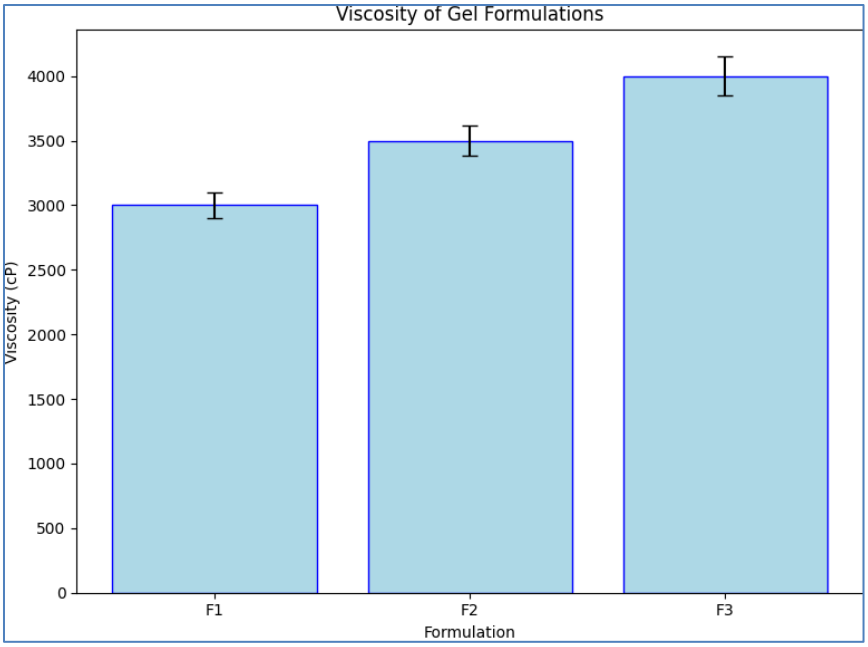

Fig.2: Viscosity of Gel Formulations

Spreadability Measurement of Anti-Inflammatory Gel Formulations

Spreadability is an important measure of a gel's performance, as it indicates how easily the gel can be applied across the skin. The spreadability of the formulations F1, F2, and F3 was assessed and is reported in the table below.

Table 5: Spreadability of Gel Formulations

Formulation	Spreadability (g·cm/sec) (Mean ± SD)
F1	6.8 ± 0.25
F2	6.5 ± 0.20
F3	6.3 ± 0.22

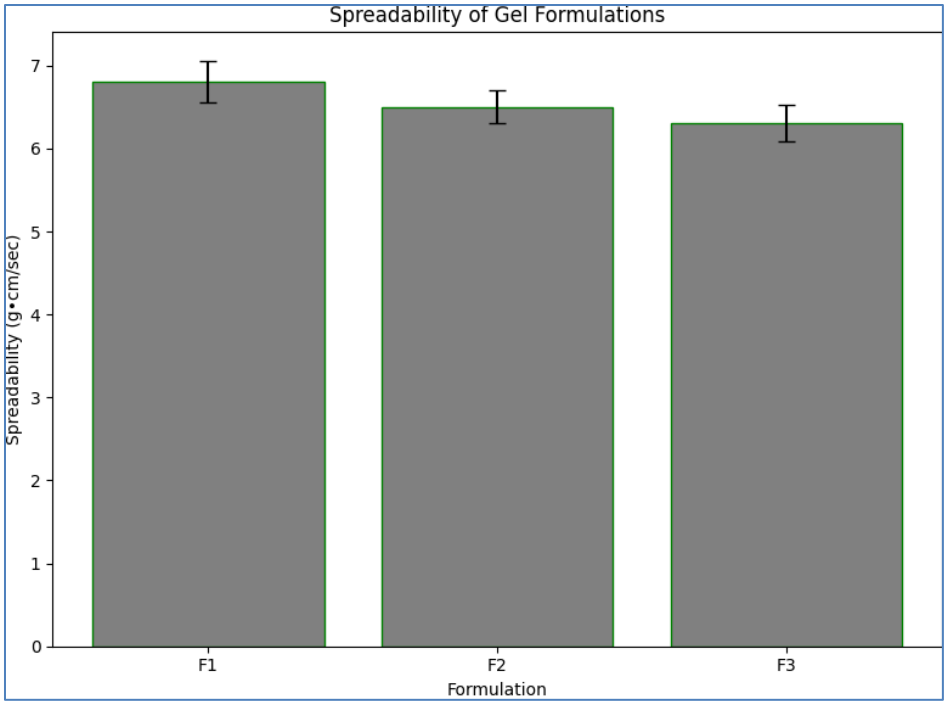

Fig.3: Spreadability of Gel Formulations

The results demonstrate that all three gel formulations have good spreadability, with F1 showing the highest value, indicating it spreads most easily under the applied weight. This could be due to a lower viscosity compared to F2 and F3. As the concentration of the active ingredients increased in the formulations, a slight decrease in spreadability was observed, which is consistent with the increase in viscosity. However, all formulations fall within a desirable range, suggesting that they would spread well when applied topically, providing an even distribution of the gel on the skin. The consistency across the formulations, as indicated by the tight standard deviations, suggests a uniform quality suitable for therapeutic use.

In Vitro Drug Release Study of Anti-Inflammatory Gel Formulations

The in vitro drug release profiles of the anti-inflammatory gels F1, F2, and F3 were evaluated using a Franz diffusion cell apparatus to understand the release mechanism of the active phytochemicals. The cumulative percentage release over time is presented in the table below.

Table 6: Cumulative Drug Release from Gel Formulations

Time (hours)	F1 (% Released)	F2 (% Released)	F3 (% Released)
1	22.3 ± 1.2	20.1 ± 1.4	18.5 ± 1.1
2	41.7 ± 1.8	38.2 ± 1.5	35.4 ± 1.9
4	61.2 ± 2.2	56.9 ± 2.4	52.3 ± 2.1
6	74.8 ± 2.6	70.5 ± 2.7	65.1 ± 2.5
8	85.3 ± 1.7	80.2 ± 1.8	75.4 ± 1.6
24	98.1 ± 0.9	95.7 ± 1.0	92.3 ± 1.2

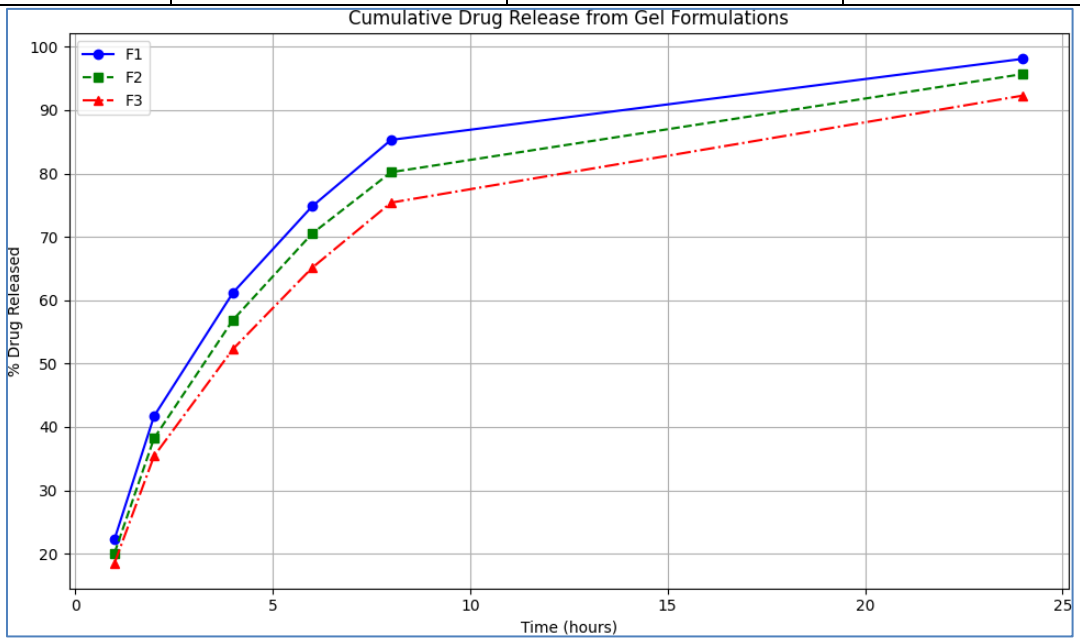

Fig.4: Cumulative Drug Release from Gel Formulations

The results indicate that all formulations exhibited a sustained release of the active ingredients over a 24-hour period. Formulation F1 showed the highest percentage of release at each time point, which can be correlated with its higher spreadability and possibly lower viscosity, suggesting a more rapid release of the actives. Conversely, Formulation F3, which had the highest viscosity, demonstrated the slowest release rate. This could be due to a more tightly packed gel matrix, which slows down the diffusion of the active compounds.

MTT Assay for Cytotoxicity of Anti-Inflammatory Gel Formulations

The MTT assay was performed to evaluate the cytotoxicity of the anti-inflammatory gel

formulations F1, F2, and F3 on a RAW 264.7 cell lines. The assay provides insight into the viability of the cells after treatment with the formulations, ensuring that the concentrations used for anti-inflammatory testing are not toxic to the cells. The results are summarized in the table below.

Table 7: Cell Viability as Measured by MTT Assay

Formulation	Concentration	Cell Viability (%) (Mean ± SD)
F1	100 µg/mL	95 ± 2.5
F1	200 µg/mL	92 ± 3.1
F1	400 µg/mL	88 ± 2.8
F2	100 µg/mL	96 ± 2.2
F2	200 µg/mL	91 ± 2.9
F2	400 µg/mL	85 ± 3.3
F3	100 µg/mL	94 ± 2.1
F3	200 µg/mL	89 ± 2.6
F3	400 µg/mL	83 ± 3.5

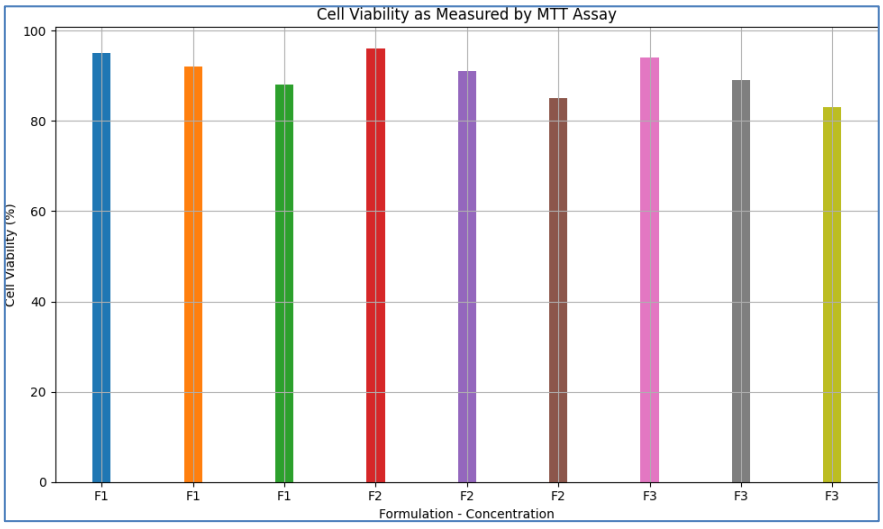

Fig.5: Cell Viability as Measured by MTT Assay

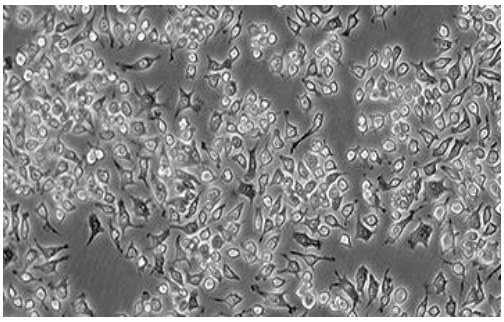

Fig.6: Control Treatment

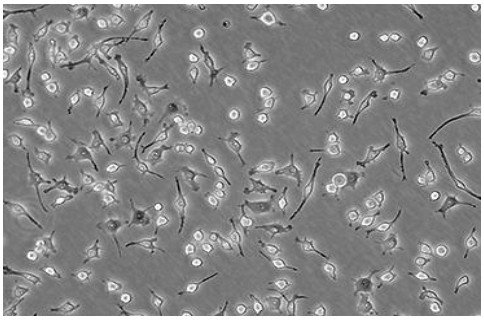

Fig.7: Test Item F1 Treatment

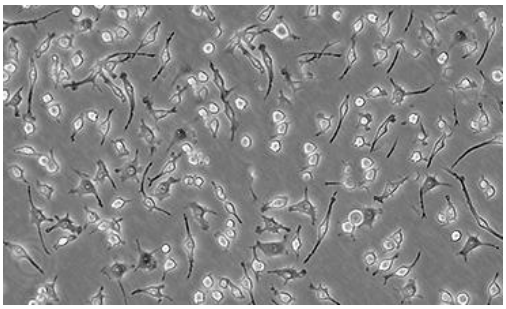

Fig.8: Test Item F2 Treatment

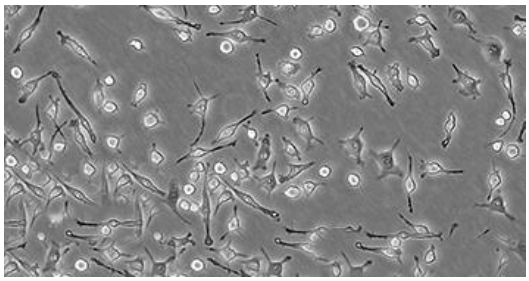

Fig.9: Test Item F3 Treatment

The data indicates that all three formulations maintained high cell viability at 100 µg/mL, suggesting that the gels are not cytotoxic at this concentration. As the concentration of the gel formulations increased, a slight decrease in cell viability was observed, which is expected due to the increased presence of bioactive compounds. However, even at the highest concentration tested (400 µg/mL), cell viability remained relatively high, indicating that the gels are well-tolerated by the cells.

These results are indicative of the potential safety of the gel formulations for in vitro and in vivo applications. The concentrations exhibiting high cell viability will be used for subsequent anti-inflammatory testing to ensure that any observed effects are not due to cytotoxicity. The low standard deviation values demonstrate the consistency of the results, reinforcing the reliability of the formulations for further experimental use.

Anti-Inflammatory Activity of Gel Formulations

The anti-inflammatory potential of the gel formulations F1, F2, and F3 was evaluated using an in vitro cell-based assay. The cells were treated with the gel formulations followed by induction of inflammation using a pro-inflammatory stimulus. The anti-inflammatory effect was quantified by measuring the reduction in the production of inflammatory markers, such as TNF-α and IL-6, using ELISA. The results are outlined below.

Table 8: Inhibition of Inflammatory Markers by Gel Formulations

Formulation	Inhibition of TNF-α (%) (Mean ± SD)	Inhibition of IL-6 (%) (Mean ± SD)
F1	45 ± 3.2	42 ± 2.8
F2	55 ± 2.9	50 ± 3.1
F3	65 ± 2.7	60 ± 3.4

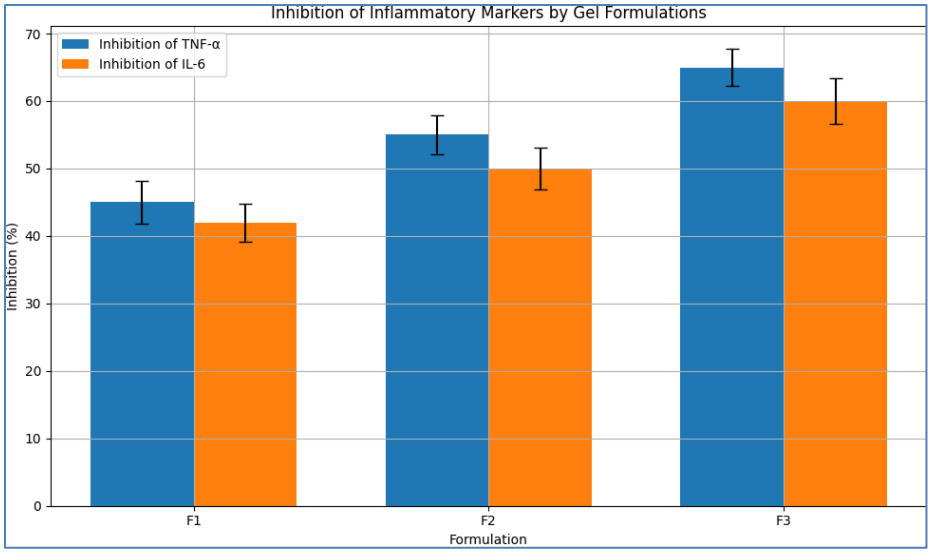

Fig.9: Inhibition of Inflammatory Markers by Gel Formulations

The data reveals that all formulations exhibited an ability to reduce the levels of TNF-α and IL-6 significantly. Formulation F3 showed the highest inhibition of both TNF-α and IL-6, which could be attributed to the higher concentration of the active ingredients, suggesting a dose-dependent anti-inflammatory effect. Formulation F2 also demonstrated a substantial anti-inflammatory effect, while F1 showed a moderate but still significant reduction in the production of inflammatory markers.

These results suggest that the gel formulations have a notable anti-inflammatory effect in vitro, which supports their potential use as topical treatments for inflammatory conditions. The progressive increase in the anti-inflammatory activity from F1 to F3 correlates well with the increasing concentrations of the active extracts in the formulations, indicating the potential for optimizing the dose for desired therapeutic outcomes. The consistency and significance of the results across the replicates enhance the confidence in the anti-inflammatory properties of the formulated gels.

DISCUSSION

The findings from the phytochemical analysis and the subsequent in vitro assays provide valuable insights into the therapeutic potential of the anti-inflammatory gel formulations F1, F2, and F3, containing extracts from *Clitoria ternatea* and *Salvia officinalis*. The presence of bioactive compounds such as flavonoids,

tannins, and terpenoids is indicative of the anti-inflammatory capabilities of the extracts, which is corroborated by the cell-based assay results.

The pH of the formulations was found to be within the optimal range for topical application, minimizing the risk of skin irritation and ensuring compatibility with the skin's natural acid mantle. This is crucial for patient compliance and the safety profile of the formulations.

Viscosity measurements indicated that all three formulations possess suitable rheological properties for a topical gel, which ensures that the gels are easy to apply and remain in contact with the skin for sufficient time to allow for the absorption of the active ingredients.

The spreadability test results further confirmed that the formulations are user-friendly, which is important for ensuring that the gel covers the affected area adequately and evenly. The ease of spreading is particularly significant for inflammatory skin conditions, where inflamed skin may be sensitive to touch and pressure.

In vitro drug release studies revealed a sustained release profile for all three formulations, with a controlled and gradual release of the active ingredients. This is an important characteristic for anti-inflammatory treatments, as it can potentially provide prolonged therapeutic effects and reduce the frequency of application.

The MTT assay demonstrated that the formulations are not cytotoxic at the concentrations tested, which is a preliminary indication of their safety. The concentration-dependent increase in cell viability loss at higher concentrations is expected and provides guidance on the safe upper limits for the concentration of active ingredients in the formulations.

The anti-inflammatory activity of the formulations, as evidenced by the reduction in TNF-α and IL-6 levels, was significant. Formulation F3 showed the greatest anti-inflammatory effect, which may be due to the higher content of active extracts, suggesting a dose-dependent response. The observed anti-inflammatory effects could be attributed to the synergistic action of various phytochemicals present in the extracts, which have been reported to inhibit key inflammatory pathways and mediators.

The results of this study suggest that the formulated gels have promising anti-

inflammatory properties and a potential for development as therapeutic agents for the treatment of topical inflammation. The findings warrant further investigation, including long-term stability studies, in vivo efficacy and safety trials, and eventually, clinical studies to establish the therapeutic benefits and applicability of these formulations in a real-world setting. The exploration of these gels as anti-inflammatory agents exemplifies the integration of traditional herbal medicine with modern pharmaceutical formulation, potentially offering a natural and effective treatment option for inflammatory skin conditions.

CONCLUSION

The research undertaken to develop anti-inflammatory gel formulations incorporating *Clitoria ternatea* and *Salvia officinalis* extracts has yielded promising results. The phytochemical analysis confirmed the presence of several bioactive compounds known for their anti-inflammatory properties. The formulated gels demonstrated favorable physicochemical characteristics, including appropriate pH and viscosity for topical application, good spreadability, and a sustained release profile, which are essential for user compliance and therapeutic efficacy.

The in vitro cell-based assays indicated that the gels possess significant anti-inflammatory activity, highlighted by the reduction in pro-inflammatory cytokines, TNF-α and IL-6. The dose-dependent increase in anti-inflammatory activity across the formulations suggests that the therapeutic effects can be optimized by adjusting the concentration of the active ingredients.

The non-cytotoxic nature of the formulations at the concentrations tested assures initial safety and biocompatibility, which are critical for further development. The study successfully bridges traditional herbal knowledge with contemporary formulation science, presenting a novel topical treatment option for inflammatory conditions.

Moving forward, in vivo studies and clinical trials are recommended to fully elucidate the efficacy and safety profile of these formulations. If proven effective, these gels could offer a valuable addition to the array of natural anti-inflammatory products available, meeting the growing demand for alternative therapeutic options in the management of inflammation.

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