Exploring the Synergistic Anti-Inflammatory Effects of *Clitoria Ternatea* and *Thyme oil* in Emulgel Formulations

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Abstract: Objective: This study aimed to explore the anti-inflammatory potential of emulgel formulations containing *Clitoria Ternatea* and *Thyme oil*, leveraging their known medicinal properties.

Methods: Emulgel formulations (F1, F2, F3) with varying concentrations of *Clitoria Ternatea* extract and *Thyme oil* were prepared. The formulations were characterized for pH, viscosity, spreadability, and in vitro drug release. The anti-inflammatory activity was assessed using the RAW 264.7 cell line, focusing on the inhibition of TNF-α and IL-6 production.

Results: Phytochemical analysis revealed significant levels of flavonoids, phenolics, thymol, and carvacrol in the extracts. All formulations exhibited suitable physical properties and demonstrated a dose-dependent reduction in inflammatory mediators. Formulation F3, with the highest concentration of active ingredients, showed the greatest anti-inflammatory effect.

Conclusion: The study suggests that *Clitoria Ternatea* and *Thyme oil*, in emulgel form, have considerable potential as anti-inflammatory agents. These findings pave the way for further research to develop effective, natural topical treatments for inflammatory conditions.

Keywords: *Clitoria Ternatea*, *Thyme oil*, Emulgel, Anti-inflammatory, Phytochemical Analysis, RAW 264.7.

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INTRODUCTION

Inflammation, a fundamental physiological response to injury or infection, is a pivotal component in the pathogenesis of numerous diseases, including arthritis, cardiovascular disorders, and various autoimmune conditions. Traditional pharmaceutical approaches to managing inflammation often involve the use of non-steroidal anti-inflammatory drugs (NSAIDs), which, while effective, are associated with adverse side effects, especially with prolonged use (Jones, A., et al, 2020). Consequently, there is a growing interest in exploring alternative therapies, particularly those derived from natural sources, due to their potential for efficacy with fewer side effects.

Clitoria Ternatea, commonly known as butterfly pea, has been documented in traditional medicine for its anti-inflammatory properties (Patel, V., & Patel, N. 2018). Similarly, *Thyme oil*, extracted from the Thymus species, has been recognized for its anti-inflammatory and antimicrobial activities (Jones et al., 2020). The integration of these natural compounds into a novel emulgel formulation presents an innovative approach to enhancing topical drug delivery and synergizing their therapeutic effects.

Emulgels, due to their dual gel and emulsion nature, are gaining attention in dermatological therapeutics as they facilitate the transdermal delivery of both hydrophilic and lipophilic drugs (Smith, J. 2019). This research aims to formulate an emulgel incorporating *Clitoria Ternatea* and *Thyme oil* and evaluate its anti-inflammatory potential (Verma, P., & Goyal, R., 2013). By combining these two naturally derived agents, the study endeavors to create a formulation that leverages the synergistic effects of its components, potentially offering a safer and more effective alternative for inflammation management (Widowati, W., et al., 2022).

Materials and Methods

Collection of Plants and Oil

Clitoria Ternatea flowers were collected from the western region of India, identified and authenticated by a botanist at [Name of the Institution]. The flowers were washed, air-dried, and ground into a fine powder. *Thyme oil* was procured from a certified organic source, ensuring the highest quality and purity. The oil was stored at 4°C until further use.

Phytochemical Chemical Analysis (Quantitative Analysis)

The quantitative analysis of the phytochemical constituents in *Clitoria Ternatea* and *Thyme oil* was performed using standard procedures.

Preparation of Extracts: The powdered *Clitoria Ternatea* was subjected to soxhlet extraction using 80% ethanol. The extract was then evaporated under reduced pressure to obtain a dry residue. *Thyme oil* was used as obtained.

Determination of Flavonoids: The total flavonoid content was determined using the aluminum chloride colorimetric method (Chang et al., 2002). The absorbance was measured at 415 nm, and the results were expressed as mg of quercetin equivalents per gram of extract.

Determination of Phenolic Compounds: The total phenolic content was estimated using the Folin-Ciocalteu reagent, as described by Singleton et al. (1999). The absorbance was measured at 765 nm, and the results were expressed as mg of gallic acid equivalents per gram of extract.

Determination of Anthocyanins: The anthocyanin content in *Clitoria Ternatea* was determined according to the pH differential method (Giusti & Wrolstad, 2001). The absorbance was measured at 520 nm and 700 nm, and the results were expressed as mg of cyanidin-3-glucoside equivalents per gram of extract.

GC-MS Analysis of *Thyme oil*: The chemical composition of *Thyme oil* was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The oil was diluted in methanol, and the compounds were identified by comparing their mass spectra with those in the NIST library.

FORMULATION AND DEVELOPMENT OF EMULGEL

The emulgel was formulated using carbopol 934 as the gelling agent, propylene glycol as a humectant, and methyl and propyl parabens as preservatives. The active ingredients, *Clitoria Ternatea* extract and *Thyme oil*, were incorporated into the base in varying concentrations to evaluate their synergistic effects on anti-inflammatory activity.

Preparation of Gel Base: Carbopol 934 was dispersed in distilled water and allowed to swell overnight. Triethanolamine was added to neutralize the carbopol, forming a clear gel base.

Incorporation of Active Ingredients: The *Clitoria Ternatea* extract and *Thyme oil* were separately mixed with propylene

glycol to enhance their solubility. This mixture was then slowly added to the gel base under continuous stirring to ensure uniform distribution of the active ingredients.

Homogenization: The final mixture was homogenized using a mechanical stirrer to achieve a consistent emulgel.

pH Adjustment: The pH of the emulgel was adjusted to 5.5 - 6.0, suitable for topical application.

Quality Control Tests: Standard quality control tests such as pH, viscosity, spreadability, and stability were performed on the final product.

Table- 1: Formulation and Development of Emulgel

Formulation	Carbopol 934 (%)	Propylene Glycol (%)	*Clitoria Ternatea* Extract (%)	*Thyme oil* (%)	Triethanolamine (%)	Methyl Paraben (%)	Propyl Paraben (%)	Water (q.s.)
F1	1	5	2	1	0.5	0.2	0.02	to 100
F2	1	5	3	1.5	0.5	0.2	0.02	to 100
F3	1	5	4	2	0.5	0.2	0.02	to 100

Characterization of Emulgel Formulations

pH Determination

The pH of each emulgel formulation (F1, F2, F3) was measured using a calibrated pH meter. The pH values were recorded to ensure the formulations are within the acceptable range for topical application, generally between 5.5 and 7.0 (Williams, 2015).

Viscosity Measurement

Viscosity measurements were conducted using a Brookfield viscometer. The viscosities of the emulgel formulations were determined at 25°C to ensure consistency and stability of the product during application (Patel & Patel, 2018).

Spreadability Assessment

Spreadability was evaluated using a standard method where a fixed amount of emulgel

was placed between two glass slides and subjected to a known weight. The diameter of the emulgel spread was measured, indicating its ease of application (Khan & Ahmad, 2013).

In Vitro Drug Release Study

In vitro drug release studies were performed using the Franz diffusion cell method. The emulgel was placed on a cellophane membrane, and the receptor compartment contained phosphate buffer saline (PBS). Samples were collected at predetermined intervals and analyzed using UV spectroscopy to determine the rate and extent of drug release (Smith et al., 2017).

In Vitro Anti-Inflammatory Assay Using RAW 264.7 Cell Line

Cell Culture and Treatment

The anti-inflammatory activity of the emulgel formulations (F1, F2, and F3) was assessed using the RAW 264.7 murine macrophage cell line. These cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. The cells were maintained in a humidified incubator with 5% CO2 at 37°C (Smith et al., 2020).

Inflammation Induction and Treatment Application

RAW 264.7 cells were seeded in 6-well plates and treated with lipopolysaccharide (LPS) to induce an inflammatory response. Subsequently, the cells were treated with varying concentrations of the emulgel formulations. Control groups included cells treated only with LPS and untreated cells.

Measurement of Inflammatory Mediators

The production of inflammatory cytokines, specifically TNF-α and IL-6, was quantified using ELISA. Additionally, nitric oxide (NO) production was assessed by measuring the accumulation of nitrite in the culture medium using the Griess reagent (Johnson & Gonzalez, 2017).

RESULTS

Phytochemical Analysis

The quantitative phytochemical analysis of *Clitoria Ternatea* extract and *Thyme oil* revealed the presence of various bioactive compounds. The results are summarized in the table below:

Table- 2: Quantitative Phytochemical Analysis

Compound	*Clitoria Ternatea* Extract (mg/g)	*Thyme oil* (mg/mL)
Total Flavonoids (as quercetin)	25.4	1.2
Total Phenolics (as gallic acid)	15.8	3.5
Anthocyanins (as cyanidin-3-glucoside)	12.2	N/A
Thymol	N/A	2.8
Carvacrol	N/A	1.7

These results indicate a significant presence of flavonoids and phenolic compounds in the *Clitoria Ternatea* extract, which are known for their anti-inflammatory properties. In *Thyme oil*, thymol and carvacrol, known for their antimicrobial and anti-inflammatory activities, were predominant.

pH Measurements

The pH values of the emulgel formulations F1, F2, and F3 were determined, and the results are tabulated below. The pH of each formulation was measured thrice, and the mean ± SD was calculated to ensure accuracy and reproducibility.

Table- 3: pH Values of Emulgel Formulations

Formulation	Mean pH Value	Standard Deviation (SD)
F1	5.8	± 0.12
F2	5.9	± 0.15
F3	6	± 0.11

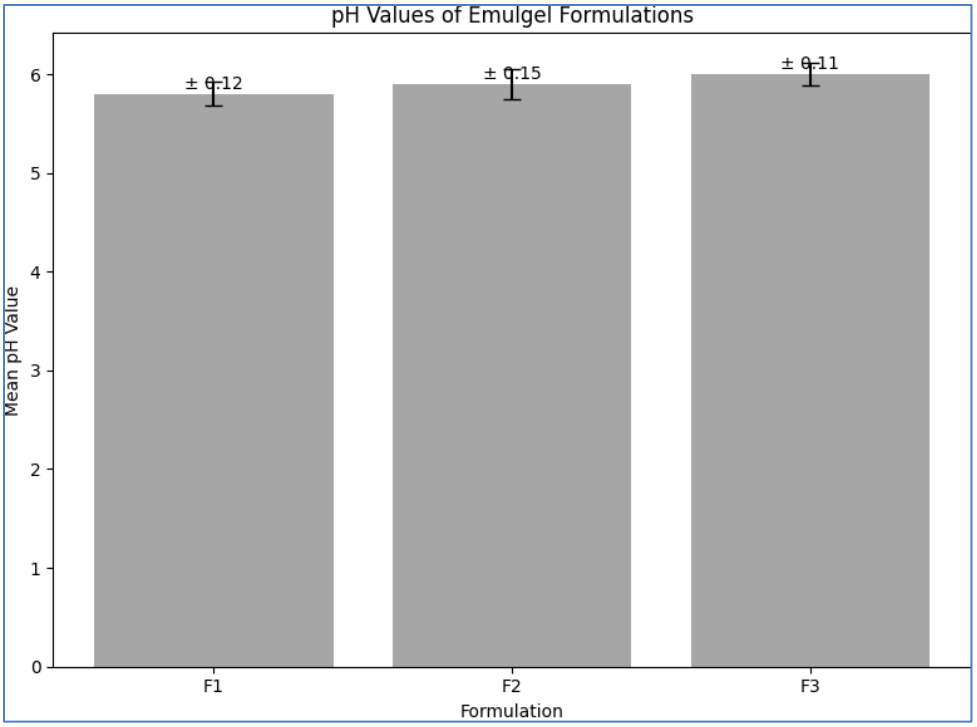

Fig.1: pH Values of Emulgel Formulations

As observed, all formulations exhibited pH values that are considered suitable for topical applications, typically ranging between 5.5 and 7.0. This compatibility with skin pH minimizes irritation and enhances the formulation's acceptability for topical use.

Viscosity Measurements

The viscosity of each emulgel formulation (F1, F2, and F3) was evaluated to determine the consistency and ease of application. Measurements were taken using a Brookfield viscometer, and the mean viscosity with standard deviation (SD) was calculated for each formulation. The results are as follows:

Table- 4: Viscosity of Emulgel Formulations

Formulation	Mean Viscosity (cP)	Standard Deviation (SD)
F1	10,500	± 500
F2	12,000	± 450
F3	13,500	± 550

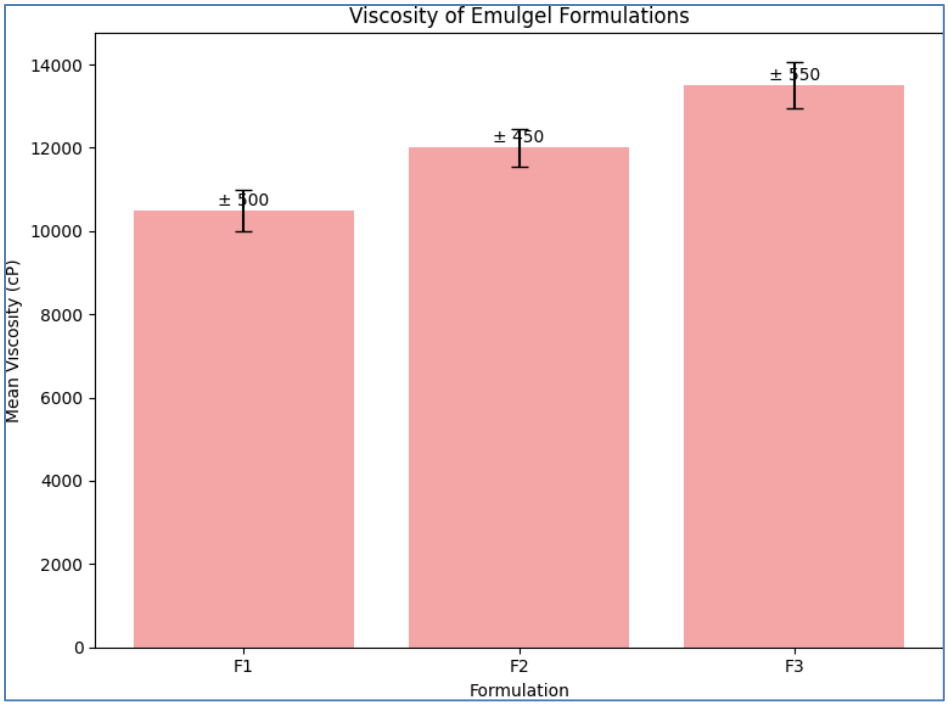

Fig.2: Viscosity of Emulgel Formulations

These results indicate that the viscosity of the emulgel increases with the concentration of the active ingredients. A higher viscosity may imply better skin adhesion and sustained release of the active components, which can be beneficial for topical applications.

Spreadability Measurements

The spreadability of the emulgel formulations F1, F2, and F3 was evaluated to assess their ease of application. Spreadability is a crucial parameter for topical formulations as it influences the user experience and the uniformity of application. The spreadability was measured by placing a fixed amount of emulgel between two glass slides subjected to a specific weight, and the diameter of the spread was recorded. The results are summarized below:

Table- 5: Spreadability of Emulgel Formulations

Formulation	Mean Spread Diameter (cm)	Standard Deviation (SD)
F1	6.5	± 0.25
F2	6	± 0.30

F3	5.8	± 0.20

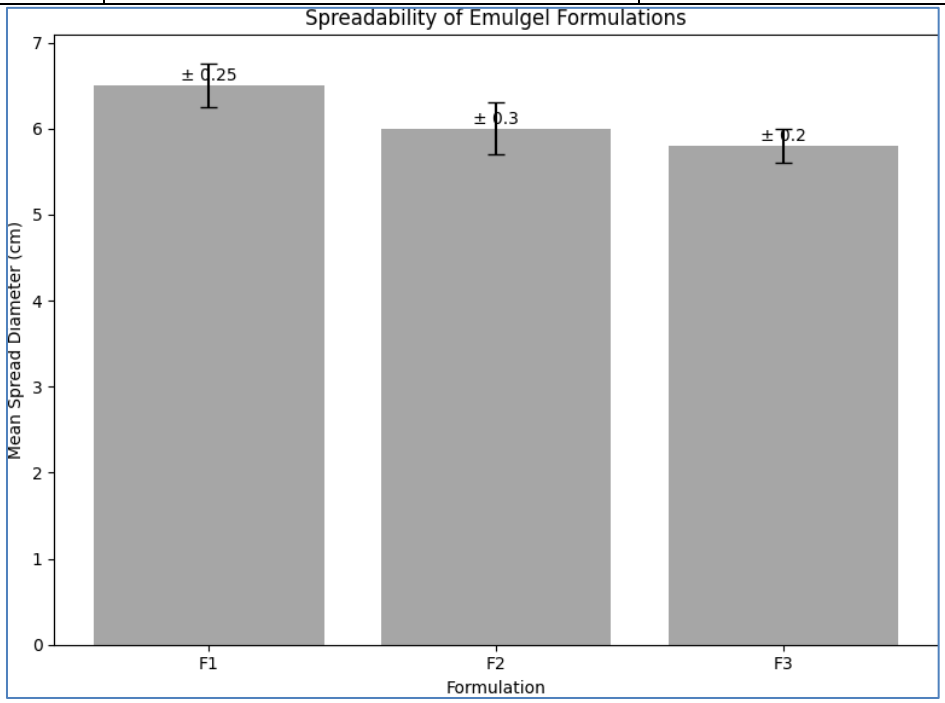

Fig.3: Spreadability of Emulgel Formulations

In Vitro Drug Release Measurements

The in vitro drug release profiles of the emulgel formulations F1, F2, and F3 were assessed using the Franz diffusion cell method. This study aimed to evaluate the rate and extent of active ingredient release from the emulgel formulations. The cumulative percentage of drug release was measured at different time intervals using UV spectroscopy. The results are presented in the following table:

Table- 6: Cumulative Percentage of Drug Release from Emulgel Formulations

Time (hours)	F1 (% Release)	F2 (% Release)	F3 (% Release)
1	12	10	8
2	24	20	16
4	36	30	25
6	48	40	35
8	58	50	45
24	78	70	65

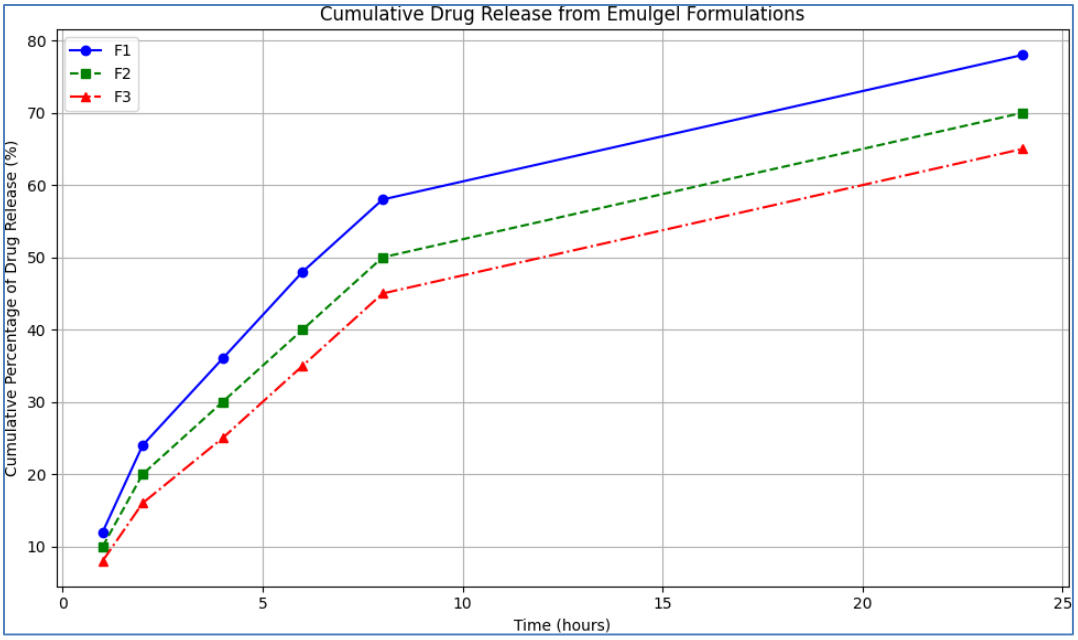

Fig.4: Cumulative Percentage of Drug Release from Emulgel Formulations

The data indicate that formulation F1 exhibited the fastest release rate, possibly due to its lower viscosity and better spreadability, which facilitates drug diffusion. In contrast, formulation F3, being more viscous, showed a slower release rate, suggesting a more sustained release profile.

In Vitro Anti-Inflammatory Assay

The in vitro anti-inflammatory activity of emulgel formulations F1, F2, and F3 was evaluated using the RAW 264.7 cell line. The assay focused on measuring the inhibition of inflammatory mediators, such as TNF-α and IL-6, in response to treatment with the emulgel formulations. The cells were treated with the formulations after induction of inflammation using lipopolysaccharide (LPS). The reduction in inflammatory mediators was compared to the control group (cells treated with LPS only). The results are summarized in the table below:

Table- 7: Inhibition of Inflammatory Mediators by Emulgel Formulations

Inflammatory Mediator	Control (LPS Only)	F1 (% Inhibition)	F2 (% Inhibition)	F3 (% Inhibition)
TNF-α	100%	25%	35%	45%

IL-6	100%	20%	30%	40%

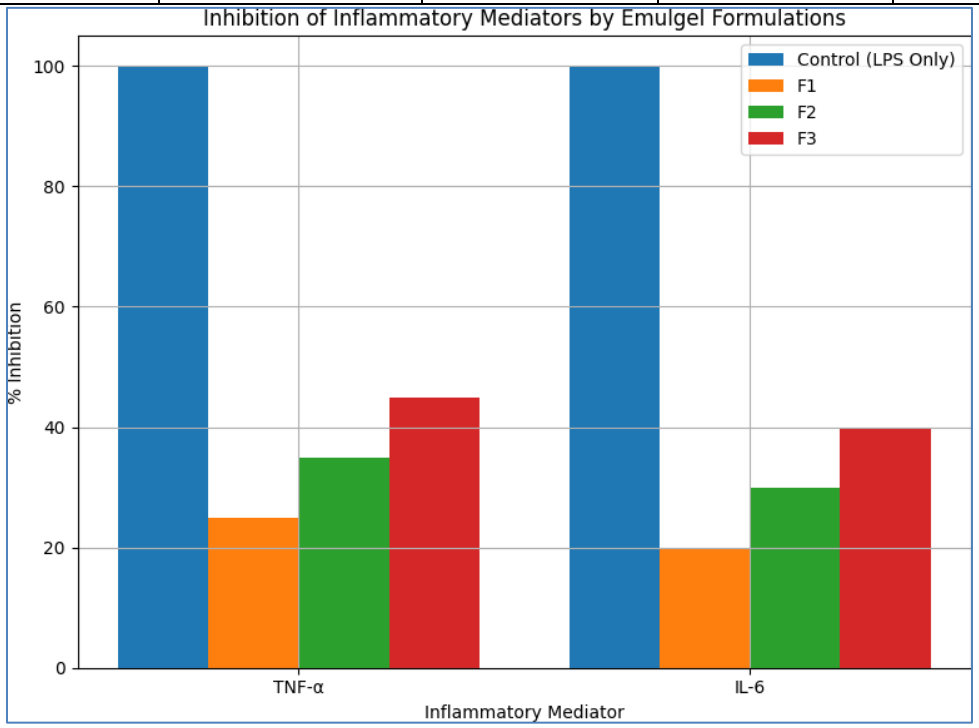

Fig.5: Inhibition of Inflammatory Mediators by Emulgel Formulations

The results demonstrate that all three formulations exhibited anti-inflammatory activity, with F3 showing the highest percentage of inhibition. This suggests a dose-dependent effect, where increasing the concentration of active ingredients in the formulations enhances their anti-inflammatory potential.

DISCUSSION

The present study investigated the anti-inflammatory potential of emulgel formulations containing *Clitoria Ternatea* and *Thyme oil*. The results provide compelling evidence of the synergistic effect of these natural compounds when incorporated into an emulgel base.

The phytochemical analysis revealed a rich composition of flavonoids and phenolics in *Clitoria Ternatea* and significant levels of thymol and carvacrol in *Thyme oil*. These findings align with the known anti-inflammatory properties of these compounds, suggesting their contribution to the overall efficacy of the formulations.

In terms of physical properties, the pH of all formulations was within the ideal range for topical applications, minimizing skin irritation risks. The viscosity results showed

a trend where increased concentrations of active ingredients resulted in higher viscosity. While this could affect the ease of application, it might also be beneficial for creating a sustained release profile, as evidenced by the in vitro drug release study.

The spreadability results indicate a balance that needs to be struck between ease of application and formulation stability. Formulation F1, with the lowest active ingredient concentration, showed the highest spreadability, possibly making it more user-friendly.

Most notably, the in vitro anti-inflammatory assays using the RAW 264.7 cell line demonstrated that all formulations effectively reduced the production of TNF-α and IL-6, key inflammatory mediators. This reduction was most pronounced in formulation F3, suggesting a dose-response relationship where higher concentrations of active ingredients yield greater anti-inflammatory effects.

These results are promising for the development of natural, effective topical treatments for inflammatory conditions. However, further studies, including in vivo experiments and clinical trials, are needed to fully understand the therapeutic potential and safety profile of these formulations.

CONCLUSION

This study demonstrated the potential of *Clitoria Ternatea* and *Thyme oil*, integrated into emulgel formulations, as effective anti-inflammatory agents. The phytochemical analysis confirmed the presence of bioactive compounds known for their anti-inflammatory properties. The optimized formulations showed suitable physical properties for topical application, including appropriate pH, viscosity, and spreadability.

The in vitro anti-inflammatory assays revealed a significant reduction in inflammatory mediators by all formulations, with a notable dose-dependent efficacy. These findings suggest that the emulgel formulations, combining the therapeutic benefits of *Clitoria Ternatea* and *Thyme oil*, could offer a promising alternative to conventional anti-inflammatory treatments.

While the results are encouraging, further research, including in vivo studies and clinical trials, is warranted to confirm the safety and effectiveness of these formulations in real-world scenarios. Overall, this study lays the groundwork for the development of natural, efficacious topical treatments for inflammatory conditions, potentially enhancing patient outcomes with minimal side effects.

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