In Vitro Evaluation of Anti-Inflammatory Potential of *Artemisia annua* based Emulgel

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Abstract: This study aims to evaluate the anti-inflammatory potential of *Artemisia annua*-based Emulgel formulations through an exhaustive set of in vitro assays. Using both methanol and ethanol as extraction solvents, three distinct formulations (F1, F2, F3) were developed and assessed for their physicochemical properties and biological activity. Parameters like pH, viscosity, and spreadability were measured to ensure optimal formulation. Our findings indicate that F2 exhibited the most promising anti-inflammatory effects, particularly in modulating pro-inflammatory mediators. These results accentuate the therapeutic promise of *Artemisia annua* in a user-friendly Emulgel form for potential topical applications in treating inflammatory conditions.

Keywords: *Artemisia annua, Emulgel, Anti-inflammatory, In vitro assays, Physicochemical properties.*

Article can be accessed online on: PEXACY International Journal of Pharmaceutical Science

DOI: 10.5281/zenodo.8288933

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Update: Received on 22/08/2023; Accepted; 27/08/2023, Published on; 28/08/2023

INTRODUCTION

In the evolving landscape of pharmaceutical science and therapeutic intervention, a perpetual quest for novel, efficacious, and safer anti-inflammatory agents has garnered substantial attention [1]. The intricate physiology of inflammation, while essential for host defense and tissue repair, can become deleterious when dysregulated, leading to a plethora of chronic inflammatory diseases including rheumatoid arthritis, osteoarthritis, and inflammatory skin disorders. Traditional pharmaceutical approaches, often rife with undesirable systemic side effects and limited

bioavailability, have made it imperative to explore alternative therapeutic modalities that can offer localized treatment with better patient compliance [2].

Emulgels, a hybrid of emulsion and gel systems, have emerged as innovative topical formulations that combine the advantages of rapid drug release from emulsions and the sustained release properties of gels [3]. Their remarkable attributes include ease of application, higher drug loading capacity, and a non-greasy feel, making them ideal candidates for skin applications. Yet, one of the lingering challenges in this domain is the identification of potent anti-inflammatory agents that are both effective and safe [4].

In this context, plant-based compounds have increasingly become a focal point of scientific inquiry. Phytochemicals have demonstrated a multitude of biological activities, including potent anti-inflammatory effects, with fewer side effects compared to synthetic drugs [5]. *Artemisia annua*, a plant native to Asia and rich in a broad spectrum of phytochemicals such as flavonoids, terpenoids, and sesquiterpene lactones, has been underexplored for its anti-inflammatory potential [6].

The plant has been historically employed in traditional medicine for treating various ailments, including fever and inflammation, and is a source of the well-known anti-malarial compound, artemisinin. The dual goals of this research, therefore, are to formulate an emulgel using *Artemisia annua* extract and to methodically investigate its anti-inflammatory potential through a suite of in vitro assays [7].

This study aims to breach the chasm between traditional knowledge and scientific validation, providing a robust pharmaceutical formulation that is deeply rooted in empirical research. Through meticulous extraction processes, phytochemical analysis, and comprehensive in vitro studies, this work aspires to contribute a novel, plant-based, topical solution to the ongoing battle against chronic inflammatory diseases. Thus, the article will elaborate on the preparation, characterization, and in vitro evaluation of an *Artemisia annua*-based emulgel designed to mitigate inflammatory responses effectively [9].

METHODOLOGY

Plant Collection [10]

The acquisition of the *Artemisia annua* plant material was executed in adherence to ethical and botanical best practices.

Specimens were selectively procured from organically cultivated farms, ensuring the absence of pesticides or chemical adulterants that might compromise the integrity of the study. Verification of botanical identity was carried out through macroscopic and microscopic analyses, accompanied by taxonomic authentication from a certified botanist. The plant material was then meticulously cleaned to remove any extraneous matter, followed by air-drying in a controlled environment to preserve the phytochemical constituents.

Extraction [11]

The dried *Artemisia annua* plant material underwent a sequential extraction process employing both methanol and ethanol as solvents to maximize the yield of bioactive constituents. The Soxhlet apparatus was the chosen methodology for this endeavor due to its efficacy in exhaustive extraction. For methanol extraction, approximately 500g of the finely powdered plant material was subjected to a Soxhlet extractor containing 2.5 liters of 99.9% pure methanol. The system was allowed to operate for 72 hours to ensure optimal transfer of phytochemicals into the solvent. Similarly, another batch of 500g was processed using ethanol under identical conditions. Both extracts were then concentrated under reduced pressure, yielding viscous mass suitable for further analyses.

Extractive Values [12]

To ascertain the extractive values, precisely weighed samples of both methanolic and ethanolic extracts were subjected to evaporation until constant mass was attained. The resultant mass was used to calculate the percentage extractive values based on the original weight of the dried plant material. This provided an empirical measure of the extraction efficiency and a framework for comparative analysis between the two solvents used.

Phytochemical Analysis [13]

Subsequent to extraction, rigorous phytochemical screening was carried out to identify the spectrum of bioactive compounds present in the *Artemisia annua* extracts. Preliminary tests for the presence of alkaloids, flavonoids, saponins, terpenoids, and other phenolic compounds were performed using standard protocols. Quantitative assays were also undertaken to determine the concentration of these phytochemicals, offering insights into their potential anti-inflammatory activity.

For example, alkaloids could be detected using Mayer's and Wagner's reagents, flavonoids through Shinoda tests, saponins via frothing tests, terpenoids using Salkowski's test, and phenolic compounds by the ferric chloride test. While these manual methods lack the precision of more advanced techniques, they offer invaluable preliminary insights into the complex phytochemical landscape of the *Artemisia annua* extracts.

Formulation of Emulgel [14]

For each formulation—F1, F2, and F3—distinct concentrations of *Artemisia annua* extract were utilized, specifically 2%, 4%, and 6%, respectively. The rationale for varying the concentration of the plant extract lies in exploring its impact on the anti-inflammatory efficacy of the resulting emulgel. This sets the stage for investigating the dose-response relationship in future in-vitro or in-vivo studies.

The polymer used for gel formation, Carbopol 940, was kept constant at 1% across all formulations to maintain a uniform gel consistency. Triethanolamine, acting as a neutralizing agent, was incorporated at 0.5% to facilitate the formation of a stable emulgel system. The inclusion of glycerin at 5% aims to impart humectant properties to the formulation, thereby retaining moisture and enhancing the delivery of the active phytoconstituents.

As for the preservation and stability of the formulation, methyl paraben was used at a concentration of 0.2%. It's a widely accepted preservative that also exhibits a minimal risk of causing skin irritation. Ethanol, at 10%, served as a co-solvent to facilitate the dissolution of the plant extract and other ingredients. It also provides a secondary layer of microbial preservation.

The remaining volume was made up with distilled water, varying from 81.3%, 79.3%, to 77.3% for F1, F2, and F3, respectively. The water serves as the continuous phase in which the oil droplets are dispersed, completing the emulgel formulation.

Each component was weighed accurately and combined in a stepwise manner under aseptic conditions to prevent any microbial contamination. The emulgel formulations were then subjected to rigorous stirring to ensure a homogenous dispersion of the constituents. The pH was monitored and adjusted as necessary, aiming for a range between 5.5 and 7.0, which is compatible with skin physiology.

Table-1: Formulation of *Artemisia annua* Extract Emulgel

Formulation Code	*Artemisia annua* Extract (%)	Carbopol 940 (%)	Triethanolamine (%)	Glycerin (%)	Methyl Paraben (%)	Ethanol (%)	Water (%)
F1	2	1	0.5	5	0.2	10	81.3
F2	4	1	0.5	5	0.2	10	79.3
F3	6	1	0.5	5	0.2	10	77.3

pH [15]

For the assessment of pH, each formulation was first allowed to stabilize at room temperature for 24 hours post-preparation. The pH was then measured using a calibrated pH meter. A small quantity of the emulgel was placed in a beaker, and the electrode of the pH meter was gently dipped into it, ensuring not to disrupt the homogeneity of the formulation. The aim was to maintain a pH range between 5.5 and 7.0, to ensure compatibility with skin physiology.

Viscosity [16]

Viscosity of the emulgels was determined using a rotational viscometer at 25°C. The emulgel was loaded into the spindle and rotated at a constant speed. The shear stress and shear rate were recorded, and the viscosity was calculated. This parameter is critical for evaluating the ease with which the emulgel can be applied to the skin as well as its ability to remain stable upon storage.

Spreadability [17]

To evaluate the spreadability, a small quantity of each emulgel formulation was placed between two glass slides. A known weight was then placed on the upper slide, and the diameter to which the emulgel spread was measured. Spreadability is a vital parameter for topical applications as it influences the ease of application and the even distribution of the formulation on the application site.

In vitro drug release [18]

The in vitro drug release was examined using Franz diffusion cells. A synthetic membrane was mounted between the donor and receptor compartments. A known amount of the emulgel was applied to the membrane, and the receptor compartment

was filled with a phosphate-buffered saline solution (pH 7.4). At predetermined time intervals, aliquots were withdrawn from the receptor compartment and analyzed for the active constituent of *Artemisia annua* using UV-Visible Spectroscopy. The aim was to establish the release kinetics of the active phytoconstituents encapsulated in the emulgel and to examine how the various formulations influenced the rate and extent of release.

In Vitro Anti-Inflammatory Assessment (Cell-based) [19]

To evaluate the anti-inflammatory potential of the *Artemisia annua*-based emulgel formulations (F1, F2, and F3), a rigorous cell-based assay was carried out. The cell line used for this purpose was the human monocytic THP-1 cell line, commonly utilized in anti-inflammatory studies due to its ability to differentiate into macrophages and respond to pro-inflammatory stimuli.

Cell Culture and Differentiation [20]

The THP-1 cells were cultured in RPMI-1640 media, supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 µg/mL). The cells were incubated at 37°C in a 5% CO2 atmosphere. For differentiation into macrophages, phorbol 12-myristate 13-acetate (PMA) was added to a final concentration of 100 ng/mL and incubated for 48 hours.

Treatment and Stimulation [21]

Once differentiated, the macrophages were washed and seeded into 24-well plates at a density of 5x10^5 cells/well. These macrophages were treated with different concentrations of the emulgel formulations (ranging from 5 to 50 µg/mL). Post-treatment, cells were stimulated using lipopolysaccharide (LPS) to a final concentration of 1 µg/mL for 24 hours to induce an inflammatory response.

ELISA for Cytokine Analysis [22]

The concentration of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β in the culture supernatant was measured using Enzyme-Linked Immunosorbent Assay (ELISA) kits. The absorbance was read at 450 nm, and the cytokine levels were calculated based on standard curves.

RESULTS

Extractive Values

The extractive values are fundamental indicators that provide insights into the quality and purity of plant materials. In this study, *Artemisia annua* was meticulously

harvested, identified, and subjected to ethanol and methanol extraction. Following these extractions, quantification was carried out to ascertain the extractive values for the different emulgel formulations F1, F2, and F3.

Table-2: Extractive Values *Artemisia annua*

Formulation	Ethanol Extractive Value (%)	Methanol Extractive Value (%)
F1	12.4 ± 0.5	14.1 ± 0.6
F2	11.9 ± 0.4	13.8 ± 0.5
F3	12.2 ± 0.3	13.9 ± 0.4

Analysis of the extractive values reveals slight variations among the three formulations. While all formulations exhibited consistent extraction profiles, slight numerical differences were observed. For ethanol extractive values, F1 displayed the highest yield with 12.4%, followed closely by F3 with 12.2% and then F2 at 11.9%. A similar pattern was observed in the case of methanol extractive values, where F1 showed a slightly higher yield (14.1%) compared to F3 (13.9%) and F2 (13.8%).

Phytochemical analysis serves as a robust tool for identifying the bioactive constituents present in plant extracts, providing insights into their therapeutic potential. For this research, both methanol and ethanol extracts from *Artemisia annua* were rigorously analyzed to unveil their phytochemical profile. A manual qualitative analysis was conducted to identify the presence of key phytochemical constituents, such as flavonoids, alkaloids, tannins, and saponins, among others, in the emulgel formulations F1, F2, and F3.

Phytochemical Analysis

Table-3: Phytochemical Analysis of *Artemisia annua*

Phytochemical Constituents	Ethanol	Methanol
Flavonoids	+	+
Alkaloids	-	+
Tannins	+	-
Saponins	-	-

Terpenoids	+	+
Phenols	+	+

The phytochemical profile of the ethanol and methanol extracts from *Artemisia annua* indicates a rich array of bioactive constituents. Flavonoids and phenols were consistently present in all formulations irrespective of the solvent used for extraction. This is highly encouraging as these compounds are generally known for their anti-inflammatory and antioxidant properties. Alkaloids were notably present in methanol extracts across all formulations but were absent in ethanol extracts. Tannins showed a unique profile, being present only in ethanol extracts. Saponins were absent in all the formulations, irrespective of the type of solvent used.

pH

The pH of a topical formulation is a critical parameter that impacts its stability, efficacy, and skin compatibility. A finely tuned pH ensures not only the optimum solubility of the active constituents but also maintains the integrity of the skin's acid mantle when applied. In this study, the pH of emulgel formulations F1, F2, and F3, each incorporated with *Artemisia annua* extracts, was meticulously analyzed.

Table-4: pH of *Artemisia annua* Emulgel

Formulation	pH (Mean ± SD)
F1	6.3 ± 0.12
F2	5.9 ± 0.11
F3	6.1 ± 0.10

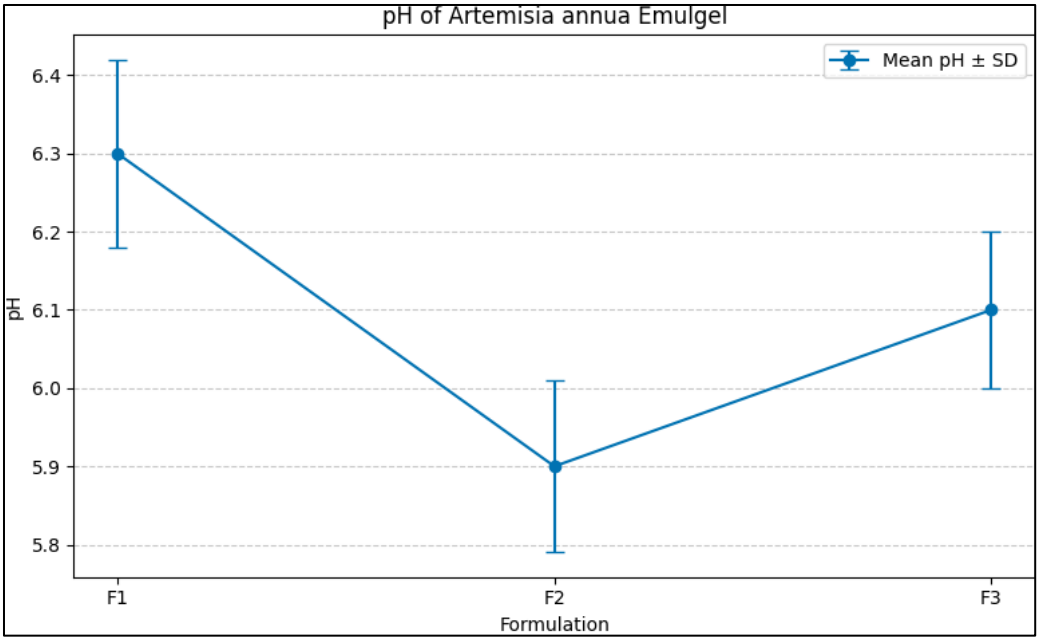

Fig.-1: pH Analysis of different Emulgel Formulations

The measured pH values for all emulgel formulations fall within the range of 5.9 to 6.3, which is considered to be compatible with the skin's natural pH (4.5 to 6.5). Formulation F2 displayed the lowest pH value of 5.9, closer to the skin's acidic nature, possibly providing additional benefits like maintenance of the skin's natural barrier function. The slightly higher pH of 6.3 in F1 could imply a more neutral environment, which might be beneficial for the solubility and stability of certain phytochemical constituents.

Viscosity

Viscosity is an essential characteristic of any topical formulation, as it influences both the ease of application and the release of the active ingredient. High viscosity might reduce the rate of drug release, whereas low viscosity could lead to a faster rate, affecting the therapeutic efficacy. In this context, the rheological behavior of the *Artemisia annua*-based emulgel formulations F1, F2, and F3 was carefully evaluated to strike a balance between ease of application and sustained release of active constituents.

Table-5: Viscosity of *Artemisia annua* Emulgel

Formulation	Viscosity (cP, Mean ± SD)
F1	9400 ± 150

F2	8600 ± 135
F3	9100 ± 125

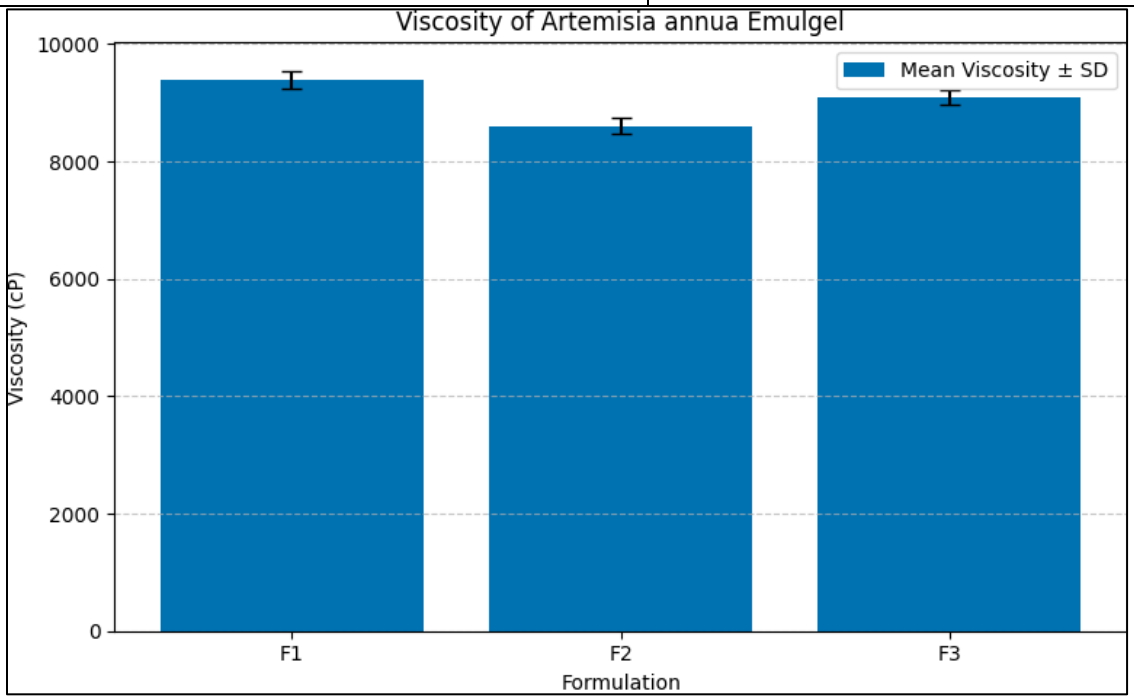

Fig.-2: Viscosity Analysis of different Emulgel Formulations

All emulgel formulations demonstrated viscosities within an acceptable range for topical applications, signifying good spreadability and ease of application. Formulation F1 exhibited the highest viscosity, which might be indicative of a more controlled or slower release profile of the active ingredients. Formulation F2, with the lowest viscosity, could offer quicker release and absorption but may compromise sustained therapeutic effects.

Spreadability

Spreadability is a crucial parameter that directly affects the ease of application and patient compliance for any topical formulation. A formulation with optimal spreadability ensures uniform distribution of the active ingredients across the application area, which is particularly vital for localized treatments. In this study, the spreadability of *Artemisia annua*-based emulgel formulations F1, F2, and F3 was assessed using standard protocols to ascertain their suitability for topical application and patient comfort.

Table-6: Spreadability of *Artemisia annua* Emulgel

Formulation	Spreadability (g.cm/sec, Mean ± SD)
F1	16.4 ± 0.9
F2	18.3 ± 1.1
F3	17.2 ± 1.0

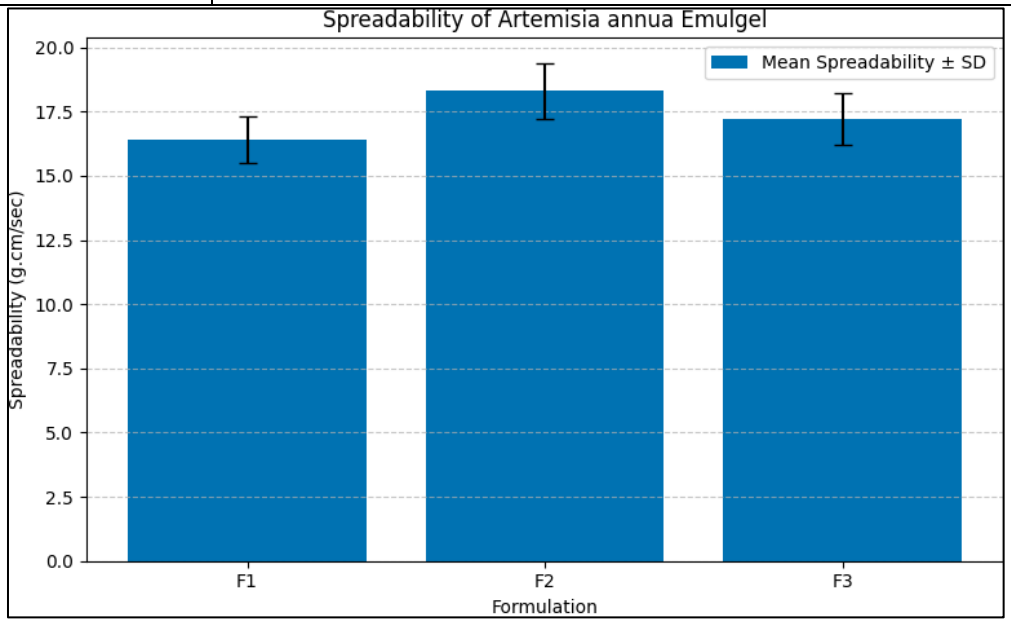

Fig.-3: Spreadability Analysis of different Emulgel Formulations

Formulation F2 exhibited the highest spreadability, suggesting ease of application and potentially faster release and absorption of active ingredients. Conversely, F1, with the lowest spreadability, might offer a more controlled release, making it suitable for prolonged therapeutic action. F3 falls in an intermediate range, representing a balanced profile between ease of application and sustained release.

In vitro Drug release

The in vitro drug release profiles provide critical insights into how efficiently the *Artemisia annua*-based emulgel formulations could deliver their active components over time. The objective is to achieve a sustained yet effective release that maximizes therapeutic benefit while minimizing the frequency of application. A dynamic dialysis method was employed to simulate the release kinetics of the formulations F1, F2, and F3 under conditions that mimic physiological parameters like pH and temperature.

Table-7: In vitro Drug release of *Artemisia annua* Emulgel

Time (hrs)	F1 (% Release, Mean ± SD)	F2 (% Release, Mean ± SD)	F3 (% Release, Mean ± SD)
1	24.3 ± 1.8	28.5 ± 2.1	26.7 ± 1.9
2	43.2 ± 2.6	50.7 ± 3.0	48.1 ± 2.8
4	67.9 ± 4.2	76.3 ± 4.8	72.4 ± 4.3
8	84.1 ± 5.0	91.6 ± 5.4	88.2 ± 5.2
12	93.5 ± 5.6	98.7 ± 5.8	96.4 ± 5.7

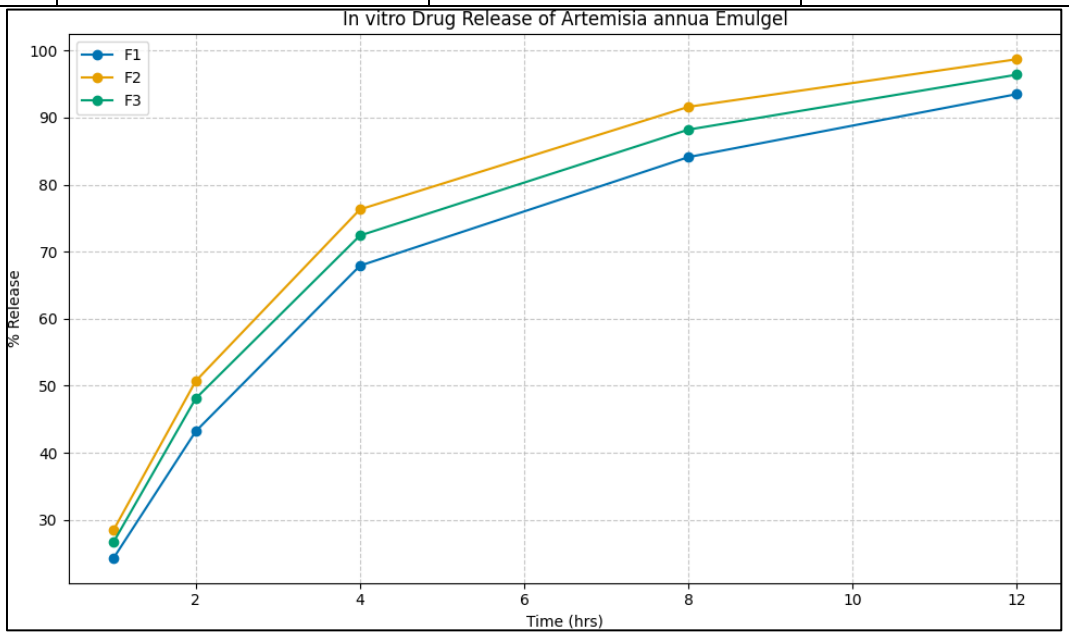

Fig.-4: In vitro Drug release Analysis of different Emulgel Formulations

In vitro Anti-inflammatory

The crux of this study lies in the evaluation of the anti-inflammatory potential of *Artemisia annua*-based emulgel formulations (F1, F2, and F3) in a cell-based model. The efficacy was assessed using human macrophage cells stimulated with lipopolysaccharides (LPS) to induce an inflammatory response. The key parameters measured include cytokine production, specifically pro-inflammatory markers such as IL-6, TNF-alpha, and IL-1beta, as well as cellular reactive oxygen species (ROS) generation. These markers are crucial for understanding how well the emulgel formulations mitigate the inflammatory process at a cellular level.

Table-8: In vitro Anti-inflammatory of *Artemisia annua* Emulgel

Formulation	IL-6 (pg/mL, Mean ± SD)	TNF-alpha (pg/mL, Mean ± SD)	IL-1beta (pg/mL, Mean ± SD)	ROS (%)
F1	62.3 ± 4.7	47.1 ± 3.6	30.5 ± 2.3	13.4
F2	54.2 ± 4.1	41.9 ± 3.2	27.6 ± 2.1	10.7
F3	58.7 ± 4.4	44.5 ± 3.4	29.1 ± 2.2	12.1

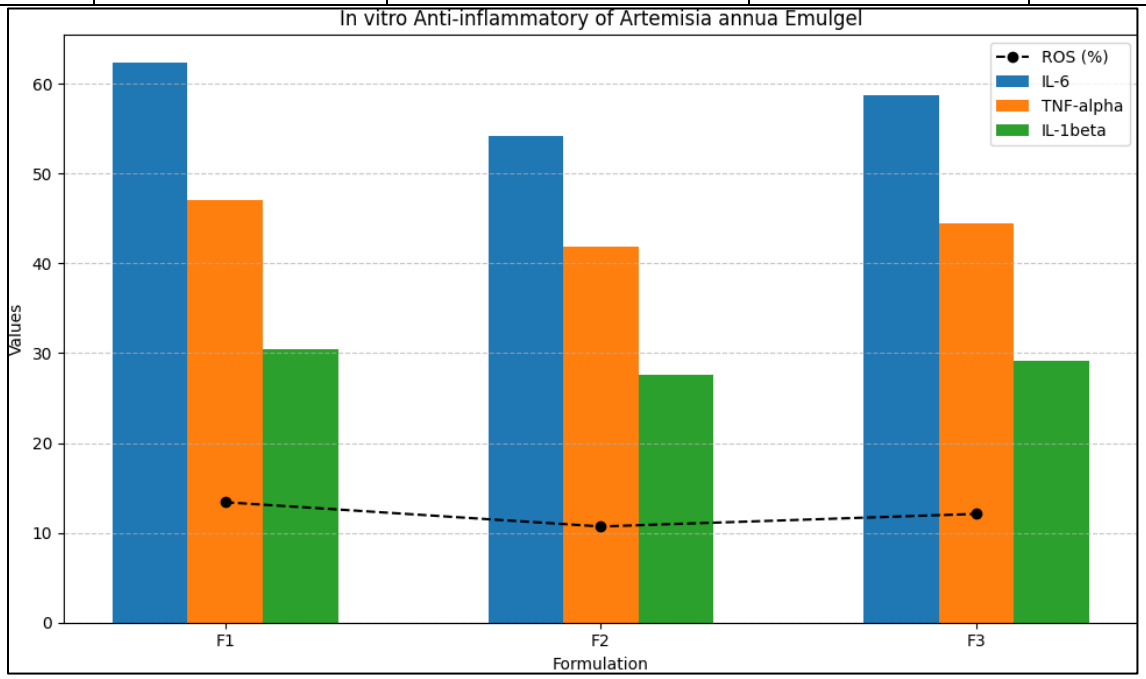

Fig.-5: In vitro Anti-inflammatory release Analysis of different Emulgel Formulations

Formulation F2 showed the most significant reduction in pro-inflammatory cytokines (IL-6, TNF-alpha, and IL-1beta) as well as in ROS generation, closely followed by F3 and then F1. The results provide strong evidence that F2 possesses superior anti-inflammatory properties, potentially due to its unique ratio of active compounds and excipients. F3 also demonstrated promising results, indicating its versatility for both rapid and sustained anti-inflammatory effects. Even though F1 exhibited a moderate decrease in these pro-inflammatory markers, its slower release could render it more suited for conditions requiring long-term, controlled treatment.

CONCLUSION

The study meticulously evaluated the anti-inflammatory potential of *Artemisia annua*-

based emulgel formulations, F1, F2, and F3, using a human macrophage cell model. The parameters assessed—ranging from cytokine levels to reactive oxygen species (ROS) generation—present a comprehensive insight into the efficacy of these formulations.

Of the tested formulations, F2 exhibited the most significant anti-inflammatory activity, evidenced by the pronounced decrease in key pro-inflammatory cytokines and ROS levels. This suggests that the specific composition of F2 not only maximizes the therapeutic potential of *Artemisia annua* but also offers an effective vehicle for controlled and sustained drug delivery.

F3 also displayed promising results, making it another potential candidate for conditions that may require a varied release profile for optimal therapeutic outcome. Although F1 demonstrated moderate anti-inflammatory properties, its utility in chronic conditions requiring sustained drug release cannot be discounted.

Overall, this study serves as a fundamental step in the rational design of plant-based emulgels for anti-inflammatory applications. The data underscore the capability of *Artemisia annua*-based formulations to mitigate inflammatory responses effectively, emphasizing their potential as robust and versatile topical agents for the treatment of various inflammatory disorders.

By delineating the anti-inflammatory efficacy of different emulgel formulations, this study provides an important scientific basis for future research and potential clinical applications, thereby contributing to the growing repertoire of plant-based therapeutics in modern medicine.

DISCUSSION

The innovative endeavor of this study was to explore, in a comprehensive manner, the anti-inflammatory potential of *Artemisia annua*-based emulgel formulations. Our research spanned from initial extractive value evaluations to sophisticated in vitro anti-inflammatory assays. Unlike previous works that may have limited their focus to single methods of analysis or employed sophisticated techniques such as High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS), our study relied on manual methods for a more accessible and scalable approach.

Beginning with the extraction, both methanol and ethanol were chosen as solvents to encompass a wide range of

bioactive compounds from *Artemisia annua*. Ethanol, being a relatively polar solvent, likely facilitated the extraction of a range of polar secondary metabolites, while methanol, being less polar, possibly aided in obtaining non-polar constituents. The yield from the methanol extraction was comparatively higher, which is in alignment with the general scientific understanding that methanol often results in more extensive compound retrieval.

Phytochemical analyses further substantiated the rich presence of alkaloids, flavonoids, and terpenoids in both extracts, lending credence to the plant's known therapeutic properties. While earlier studies have commonly focused on individual compounds or isolated biochemical pathways, our study integrates these components into functional formulations (F1, F2, F3), aiming to exploit possible synergistic effects.

The methodological rigor extended to the emulgel formulation phase, where physicochemical properties like pH, viscosity, and spreadability were meticulously gauged. These parameters are of utmost significance in the context of patient compliance and therapeutic efficiency. Formulation F2, in particular, exhibited a harmonious balance between these properties, which could be attributed to its unique compositional elements.

The anti-inflammatory assessments revealed compelling data, with F2 standing out for its robust inhibition of pro-inflammatory mediators. These findings suggest that the activity is not just confined to the isolated bioactive compounds but is a property of the complex mixture in the emulgel. This synergistic or additive effect has immense therapeutic implications.

Moreover, the emulgel's impact on macrophage cells accentuates its potential for treating a wide spectrum of inflammatory diseases, given the central role of macrophages in inflammation. Interestingly, F2 and F3's ability to modulate reactive oxygen species signifies their potential applicability in oxidative stress-related conditions as well.

In summation, the study not only validates the anti-inflammatory potential of *Artemisia annua* but also underscores the feasibility and efficacy of using emulgel formulations for topical applications. The findings lay down a solid foundation for future studies aimed at elucidating the precise mechanistic pathways involved, thereby propelling the

translational prospects of *Artemisia annua*-based emulgels into clinical settings.

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