

Formulation and evaluation of anti-inflammatory gel using plant based bioactive compounds.

Sangram S. Shinde, Dr. Sunita S. Deore

Department of Pharmaceutics, School of Sciences, Sandip University, Nashik, Maharashtra
422213, India.

Corresponding Author:

Sangram S. Shinde

Department of Pharmaceutics, School of Pharmaceutical Sciences, Sandip University,
Nashik, Maharashtra 422213, India

ABSTRACT-

Inflammation is used by the body as a form of defence mechanism. When the inflammation persists for an extended period, it causes harm to our body tissues and creates many problems. The use of drugs such as -steroid Anti-Inflammatory Drugs, also known as NSAIDs, is common when dealing with inflammation. Even though they are effective, continued use results in side effects.

The aim of this study was to manufacture a gel that helps with inflammation using natural ingredients from plants such as *Curcuma longa* (curcumin), *Camellia sinensis* (plant source of green tea), *Withania somnifera* (ashwagandha), and *Aloe barbadensis* (plant source of aloe vera). In order to extract the plant material, a method called Soxhlet extraction was used. Then the plant material was added to a gel form called Carbopol-940.

The manufactured gel was then tested for pH levels, consistency, spreadability, homogeneity, and uniform distribution of drug.

The gel was also tested for effectiveness against inflammation through proteins.

It was discovered that the optimal gel variant possesses skin-beneficial qualities. The drug's pH level is optimal, as it disperses effectively across the skin, ensuring even distribution.

The gel underwent testing to observe its temporal alterations. The findings indicate minimal alteration, which is indeed a matter. This novel cream effectively prevented protein damage. Consequently, it could be highly effective in diminishing inflammation. Findings indicate that this plant-based gel might be a secure method for addressing skin swelling. Utilizing a plant-derived gel could serve as a substitute for alternative medications in treating inflammation in certain body regions. This botanical gel shows great potential in diminishing inflammation.

Keywords: Plant-derived gel, Curcumin, Extract of Green tea, Carbopol 940, Anti-inflammatory properties, Surface composition.

INTRODUCTION-

Inflammation occurs as our body attempts to repair itself following adverse events such as an infection. People subject themselves to pain at times. Humans have a way of protecting themselves. Inflammation is one aspect of this. It helps get rid of the problem once and for all. This helps the body recuperate after that. Sometimes, it could be out of control, causing problems like arthritis, skin problems, and more inflammations. These conditions can become really severe, particularly in cases where there is continuous inflammation, making management really important. There are various types of drugs, known as NSAIDs (non-steroidal anti-inflammatory drugs), prescribed by doctors to people suffering from inflammation and pain. Long-term use of NSAIDs poses various health risks. For example, NSAIDs can pose various health complications like stomach and intestinal complications, kidney complications, and heart complications. These problems associated with NSAIDs have made it necessary for people to look for other safer options.

There is great interest among patients for the use of drug delivery systems in managing inflammatory conditions. The efficiency in this method is due to the ability of the drug delivery system to deliver the medicine directly to the target site. Using such systems allows for non-absorption of the drug in the whole body.

While various topical drugs exist, gels are the preferred choice for many. The appeal of gels lies in their smooth texture, ease of application, and effective spreading. These are favored by patients. Gels, a type of topical drug delivery system, stand out due to their tripartite structure, capable of containing substantial water without disintegrating. Gel-based medication is administered straight to the impacted area, explaining the widespread popularity of topical drug delivery systems. For systems delivering topical drugs, gels are an option. Polymers from Carbopol excel in enhancing product thickness. My reference is to Carbopol polymers, such as Carbopol 940. The Carbopol 940 polymers are frequently utilized to increase thickness due to their exceptional quality. Their clarity is also evident. Their decomposition is not a simple process. Hence, the effectiveness of Carbopol polymers in interacting with various bioactive substances.

Plants with medicinal properties are beneficial as they contain numerous elements that can enhance our well-being. These plants contain elements that aid in reducing inflammation and can also prevent certain cells from being harmed. This quality renders them ideal for applying creams and similar products to our skin.

Curcumin stands as one of these items. Its origin is a plant known as *Curcuma longa*. Curcumin effectively halts certain inflammatory responses, such as those caused by the overactive cyclooxygenase-2, nuclear factor kappa B, and pro-inflammatory cytokines. Curcumin, sourced from *Curcuma longa*, is truly beneficial in this matter. Known alternatively as *Camellia sinensis*, green tea contains a component referred to as polyphenols. Epigallocatechin gallate, a type of polyphenol, excels in combating harmful substances that harm our skin and aids in repairing damaged skin. The combined use of multiple plant-derived natural substances may contribute to enhanced anti-inflammatory activity. This is because these bioactive components have the ability to exert their effects on different pathways of inflammation at once. The extraction process should be done properly for one to extract these bioactive components from the plant material.

One of the best ways that can be used to extract these components from the plants is using the Soxhlet technique. This technique would be very effective when dealing with plants that have the proper solvent

Making the extract a gel would ensure that the active component will be released gradually.

This allows natural substances in plants to work better

The plant-based stuff is good and when it is used with together, it helps with inflammation. People want alternatives to synthetic anti-inflammatory drugs. So it did a study to make and test a plant-based -inflammatory gel. This gel has extracts of *Curcuma longa*, *Camellia sinensis*, *Withania somnifera* and *Aloe barbadensis* in it. It has checked the plant-based -inflammatory gel to see what it is, like and if it is stable. Formulation also tested the plant-based -inflammatory gel to see if it can reduce inflammation. The main goal of the study was to see if the plant-based anti-inflammatory gel can be used to treat localized conditions. The plant-based anti-inflammatory gel seems to be a treatment option.

3. METHODOLOGY

3.1 Materials

The material extract from plants is turmeric, also known as rhizome, camellia, which, like the tea leaves of *Withania somnifera*, is also called aloe vera. used Cabo 940 to make the mixture as thick as gel.

Additionally, propylene glycol and glycerin were employed to maintain the blend's moisture and enhance its penetration into the skin. TEA, also known as Triethanolamine, is employed to maintain the mixture's acidity and base level.

To maintain the freshness of the blend and inhibit its growth, methyl paraben and propyl paraben are employed. ensured the purity of every liquid and chemical used.

3.2 Extraction of Plant-Based Bioactive Compounds

3.2.1 Extraction of Curcumin (*Curcuma longa*)

Transferred 10 grams of turmeric powder into a unique vessel known as a cellulose thimble. Subsequently, Then inserted the container into a machine to aid in extracting the beneficial elements from the turmeric. employed a blend of ethanol and propylene glycol for extraction. The turmeric was immersed in the blend for a duration of 6 hours. Subsequently, employed a different device to eliminate the liquid. This device, known as a vaporizer, functioned within a temperature range of 35 to 40 degrees Celsius. Ethyl acetate was used to purify the residue. Next, cleanse using water. Subsequently, add brine. Subsequently, the curcumin extract undergoes drying using sodium sulfate, known as anhydrous sulfate or sodium sulfate, to eliminate surplus moisture. Ultimately, the curcumin extract underwent concentration, resulting in the acquisition of the raw curcumin extract.

3.2.2 Extraction of Green Tea (*Camellia sinensis*)

Initially, dry tea leaves undergo a 48-hour immersion in petroleum ether to eliminate fat content. Next, dehydrate the green tea leaves that are fat-free. The green tea leaves are then subjected to a process called sorbet extraction. The process used 96% ethanol as a solvent to extract the material from green tea leaves. The extraction process lasted four to 6 hours. It stopped when the liquid in the tube went colourless. The green tea extract was then thickened

with an an evaporator. tea extract can be obtained by keeping the temperature below 45 degrees Celsius. The outcome yielded a tea extract derived from the leaves of green tea.

3.2.3 Extraction of Aloe Vera (*Aloe barbadensis*)

Washed the aloe very well. Then separated the parts from the internal gel part. The outer layer dries at a certain temperature until completely dry. It is then made into powder. They took 10 grams of the powder and used a special machine to remove the good stuff. They do it with a liquid called ethanol. It took around 4 to 6 hours to get it out. When they did, they got rid of the liquid and saved a good portion of it, the so-called ethanol extract aloe vera, in a special brown container. They left it there until they needed to use aloe vera extract.

3.2.4 Extraction of Ashwagandha (*Withania somnifera*)

Ten grams of ashwagandha root powder were used for extraction. The material was subjected to ethanol extraction using suitable apparatus for approximately four to six hours. Following the extraction process, the ethanol was removed to obtain the crude extract. Further purification was carried out using ethyl acetate, after which the extract was washed with distilled water and brine solution. The resulting extract was then dried using a drying agent and subsequently concentrated to obtain a purified form of ashwagandha extract.

3.3 Formulation of Anti-inflammatory Gel

For the mixture, two grams of Carbopol 940 were combined with 140 millilitres of distilled water. Continuous stirring was applied to the mixture. Initially, dry tea leaves undergo a 48-hour immersion in petroleum ether to eliminate fat content from the tea. Next, dehydrate the green tea leaves that are fat-free. This led to the creation of a tea extract derived from the leaves of green tea. This was done for one hour so that Carbopol 940 could absorb the water properly. Methyl paraben and propyl paraben were then added to this mixture. First 0.36 grams of Methyl paraben and 0.04 grams of paraben were dissolved in 5 milliliters of ethanol. The ethanol was warmed up a little to help Methyl paraben and propyl paraben dissolve. Then this mixture of Methyl paraben and propyl paraben was added to the Carbopol 940 mixture.

The mixture was prepared by initially taking a suitable container, in which propylene glycol (10 g) and glycerine (6 g) were combined. Subsequently, the previously prepared plant extracts were incorporated, including curcumin extract (3 g), green tea extract (3 g), aloe vera extract (4 g), and ashwagandha extract in appropriate quantity. The mixture was continuously stirred to ensure uniform mixing of all components.

Thereafter, the prepared mixture was added slowly to the Carbopol dispersion with constant stirring to obtain a homogeneous formulation. Then added Triethanolamine, which is also known as TEA to the mixture a little at a time. This was done to get the pH right, between 5.5 and 6.5. When they did this a gel started to form. Subsequently, distilled water was introduced to reach a total volume of 200 grams. Subsequently, they thoroughly blended everything to ensure uniformity in the Triethanolamine gel.

3.4 Evaluation of Gel Formulation

3.4.1 Physical Appearance and Homogeneity

It looked at the gel to see what color it was. If it was clear, also checked if the gel was consistent. To see if the gel was mixed well Then took a bit of the gel and put it between two glass slides. Then It looked closely to see if there were any big particles, in the gel or if it was starting to separate into different parts.

3.4.2 Determination of pH

A sample of gel weighing 1 g was accurately taken and transferred into a suitable container. This was done by adding 10 mL of distilled water and allowing it to disperse evenly to form a homogeneous solution. Dispersion of the solution was stirred gently to make sure that the gel was mixed homogeneously with the aqueous solution.

The pH of the solution was measured using a digital pH meter calibrated properly. Before measuring the pH, it was standardized using suitable buffers. The electrode was dipped in the sample solution, and the value was noted when a steady reading was observed.

3.4.3 Viscosity Measurement

To measure the gel's thickness, It employed a device known as a Brookfield viscometer. The instrument aided in determining the gel's thickness. The tool was adjusted to match the gel's speed and temperature.

Formulation did this test three times to make sure we got the answer. Then wrote down the result and included the standard deviation to show how much the results varied. Then did all these tests, on the gel to find out its viscosity.

3.4.4 Spreadability

This used a way to figure out how well something spreads. This is called the plate method. put an amount of gel between two glass plates. Then put a weight on top for a while. After that measured how far the top plate moved. To find out the spreadability of the gel used a formula with the distance the plate moved. The spreadability of the gel is very important. calculated the spreadability of the gel using this method and the formula.

The formula for work is very simple. It is calculated by multiplying the mass that is applied and the length that is moved and then dividing that by the time that is taken. So the formula S which's equal, to the applied mass, which call M multiplied by the length moved which call L . Then it divide that by the time taken which we call T . This gives us $S = \frac{M \times L}{T}$ the applied mass M multiplied by the length moved L . Then it divides that by the time taken T .

3.4.5 Drug Content Uniformity

One gram of gel was dissolved in a solvent and then filtered. This was done to get a liquid. The solution was then analyzed using a machine that can detect the amount of drug in it. This machine is called UV- spectrophotometry and it was set to the right wavelength to find out how much drug was, in the gel.

3.4.6 In-vitro Anti-inflammatory Activity (Protein Denaturation Method)

Tests were conducted on the gel to determine its effectiveness in diminishing inflammation. This was done by using a method that involves egg albumin. A mixture was made with egg albumin, a kind of buffer and the gel that was being tested. This mixture was then heated to a high temperature, 70 degrees, for 5 minutes. After it cooled down a special machine called a UV- spectrophotometer was used to measure how much of the mixture was absorbed. The gel was compared to a drug called Diclofenac sodium. The gels ability to prevent the egg albumin from breaking down was calculated as a percentage. This is important because the gels anti-inflammatory activity is being evaluated. The anti-inflammatory activity of the gel is what is being looked at.

3.4.7 Stability Studies

The gel That made was put into containers and kept at a cool temperature like you find in a refrigerator, which is around four degrees Celsius. It was also kept at room temperature, which is twenty-five degrees Celsius and at a really high temperature about forty degrees Celsius to see what would happen. It looked at the samples from time to time to see if the gel had changed in any way like if it looked different or if its pH or thickness had changed.

Table: Comparative Formulation of F1, F2 and F3 Batches of Multi-Herbal Anti-Inflammatory Gel

Sr. No.	Ingredient	F1 (g)	F2 (g)	F3 (g)	Role
1	Turmeric Extract	2	3	4	Anti-inflammatory
2	Green Tea Extract	2	3	4	Antioxidant
3	Aloe Vera Extract	3	4	5	Soothing Agent
4	Ashwagandha Extract	2	3	4	Adaptogenic Anti-inflammatory
5	Carbopol 940	2	2	2	Gelling Agent
6	Propylene Glycol	10	10	10	Penetration Enhancer
7	Glycerin	6	6	6	Emollient
8	Methyl Paraben	0.36	0.36	0.36	Preservative
9	Propyl Paraben	0.04	0.04	0.04	Preservative
10	Triethanolamine	q.s.	q.s.	q.s.	pH Adjuster
11	Distilled Water	q.s. to 200 g	q.s. to 200 g	q.s. to 200 g	Vehicle

Three different batches of multi-herbel anti-inflammatory gels (F1, F2 and F3) were prepared by altering the concentration of plant-based bioactive extracts while maintaining the concentration of the adjuncts. Lot F1 had lower concentrations of herbal extracts, Lot F2 had medium concentrations and Lot F3 had higher concentrations. Preparation of multiple batches was aimed at studying the physicochemical characteristics, drug content, diffusion, viscosity, and anti-inflammatory potential of all formulations for the purpose of selecting the best formula.

Table: Composition of Multi-Herbal Anti-Inflammatory Gel (200 g Batch)

Sr. No.	Ingredient	Quantity	Role
1	Turmeric Extract (Soxhlet)	3g	Active Anti-inflammatory
2	Green Tea Extract (Soxhlet)	3g	Antioxidant/Anti-inflammatory
3	Aloe Vera Liquid Extract	4g	Soothing Agent/Vehicle
4	Carbopol 940	2g	Gelling Agent
5	Propylene Glycol	10g	Humectant/Penetration Enhancer
6	Glycerin	6g	Emollient
7	Methyl Paraben	0.36g	Preservative
8	Propyl Paraben	0.04g	Preservative
9	Triethanolamine (TEA)	q.s. (~1g)	pH Neutralizer
10	Distilled Water	q.s. 200g	Solvent/Vehicle

4. RESULTS AND DISCUSSION

Properties of the herbal gel, which has an anti-inflammatory effect were investigated. What effect it has. The effect of applying this herbal anti-inflammatory gel on the skin was examined as well as the stability of the gel itself. Properties of the herbal anti-inflammatory gel were studied in order to ascertain whether it can be applied to the skin. Whether the herbal anti-inflammatory gel was suitable for application on the skin? Results of this study are presented below.

4.1 Physical and Organoleptic Characteristics

The gel prepared was very smooth. It had a uniform texture. There were no lumps or any abnormal distinct regions present in the sample. The color of the sample was a light to dark amber/brown color. The reason behind this is the presence of curcuminoids and green tea extracts in the preparation. The texture was not rough at all but rather very uniform. The presence of a uniform texture is indicative of proper hydration of the polymer Carbopol 940 and incorporation of plant extracts. This is favorable for cosmetic products as these formulations are always preferred by individuals. It is due to their applicability. The gel is a formulation hence; it needs to have favorable characteristics for its usage.

4.2 Physicochemical Evaluation

The following physical parameters of the obtained preparations were studied: pH, viscosity, spreadability, homogeneity, appearance, and content of drug substances.

According to the research results, the pH value of the modified preparation F2 was 6.2 ± 0.1 . The pH corresponds to the normal physiological value for the skin (5.5–6.5), which means that the preparation can be used on the skin, causing minimal irritative or sensitizing effect on it. The correct pH regulation with the use of triethanolamine helped to create a Carbopol gel system.

The modified preparation had the viscosity value of $32,561 \pm 420$ cP, The maintenance of proper viscosity is vital for topical formulations because it ensures prolonged retention and prevents easy flow off from the skin surface.

The spreadability recorded was found to be 22.32 ± 1.1 g·cm/s, indicating a well-spread composition on the skin surface. Improved spreadability ensures greater compliance from the patients and helps ensure that the formulation spreads evenly in correlation with the affected area. The use of propylene glycol and glycerine helped improve the softness of the gel.

The homogeneity of drug content was found to be 96.15 ± 1.2 %, thereby suggesting a homogeneous distribution of the natural biologically active substance within the gel. This indicates that the extraction and mixing process of herbal extracts with the gel composition was successful and consistent.

Among the three batch formulations (F1, F2, and F3), the batch formulation F2 had the most homogenous physical and chemical properties and better efficacy. Therefore, formulation F2 was selected as the optimized formulation for further analysis via FTIR, DSC, diffusion, anti-inflammatory, and stability.

Table: Comparative Evaluation of F1, F2 and F3 Formulations

Parameters	F1	F2	F3
pH	5.8 ± 0.1	6.2 ± 0.1	6.6 ± 0.2
Viscosity (cP)	$28,540 \pm 350$	$32,561 \pm 420$	$38,240 \pm 510$
Spreadability (g·cm/s)	18.45 ± 0.8	22.32 ± 1.1	16.72 ± 0.9
Drug Content (%)	91.84 ± 1.5	96.15 ± 1.2	94.72 ± 1.4
Homogeneity	Good	Excellent	Good
Appearance	Slightly thin	Smooth & uniform	Slightly thick

Of the three formulations developed, F2 was the one with the best physicochemical properties. Batch F2 had a pH of 6.2, falling within the permissible pH values for the skin; hence it would be safe to apply externally without any irritation effects. Batch F2 had the most appropriate viscosity that made it easier for application and retention at the administration site. Batch F2 also had better spreadability compared to batches F1 and F3, making it easier for patients to

apply. F2 had the best drug content uniformity, implying that the biologically active substances derived from plants were uniformly distributed throughout the gel matrix.

Table: Physiochemical Evaluation of Optimized Batch (F2)

Appearance	Observed Value (Mean $\pm$ SD, n=3)	Specification
Appearance	Smooth, Homogeneous	-
Colour	Amber -Orange	Characteristic
pH	6.2 $\pm$ 0.1	5.5-6.5
Viscosity	32,561 $\pm$ 420 cP	30,000-60,000 cP
Spreadability	22.32 $\pm$ g.cm/s	>5 g $\cdot$ cm/s
Drug Content	96.15 $\pm$ 1.2%	90 -110%

4.2.1 Fourier Transform Infrared Spectroscopy (FTIR) Study

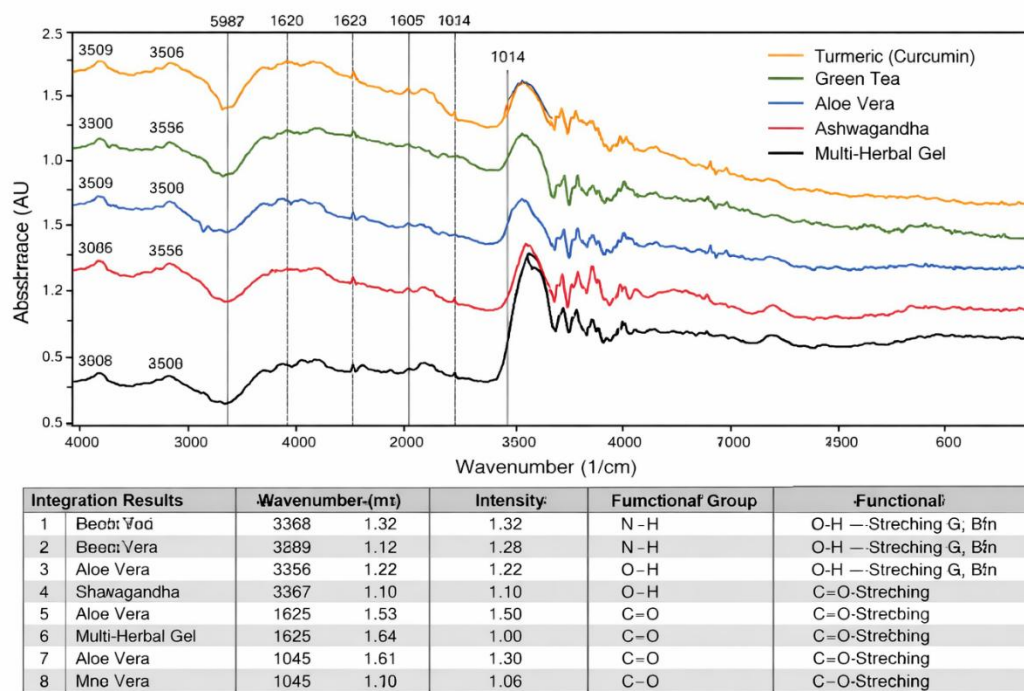
In addition, the possibility of interaction between the selected bioactive compounds of plants and the components used in the preparation of the anti-inflammatory gel formulations was tested using FTIR analysis. In the present study, the FTIR analysis of the pure extracts and the optimized gel formulation was performed. The major peaks corresponding to curcumin, green tea extract, aloe vera, and wormwood were identified.

Table: FTIR Interpretation of Optimized Gel Formulation (F2)

Functional Group	Characteristic Peak (cm ⁻¹)	Interpretation
O–H Stretching	3200–3500	Phenolic compounds and alcohol groups
C=O Stretching	1620–1700	Carbonyl group of curcumin
C=C Aromatic Stretch	1500–1600	Aromatic ring present
C–O Stretching	1000–1300	Alcohols and ethers
N–H Stretching	3300–3500	Proteinaceous compounds

FTIR spectra of the optimized F2 formulation showed all the main characteristic peaks related to functional groups of extracts of plants with no significant peak shifting or loss. Broad bands seen in the range from 3200 to 3500 cm⁻¹ denote O-H stretching of phenolic functional groups in extracts like curcumin, green tea polyphenols, and aloe vera. The band found in the range from 1620 to 1700 cm⁻¹ denotes C=O stretching of curcumin and other extracts. Bands seen at 1000 to 1300 cm⁻¹ indicate C-O stretching, which shows the presence of alcohol and ether. No significant peak shifting indicates no drug-except interaction and extract-Carbopol compatibility.

Figure: FTIR Spectrum of Optimized Multi-Herbal Anti-Inflammatory Gel (F2)



4.2.2 Differential Scanning Calorimetry (DSC) Study

The study of compatibility of plant-based bioactive compound with excipient utilized for optimizing gel formulation was performed using Differential Scanning Calorimetry (DSC). The results of DSC analysis may reveal any potential interactions of active compounds with polymer gel through changes in melting point, shifting of peaks, and absence of distinct thermal peaks. The thermal profile of pure extract and optimized gel formulation was analyzed for compatibility assessment.

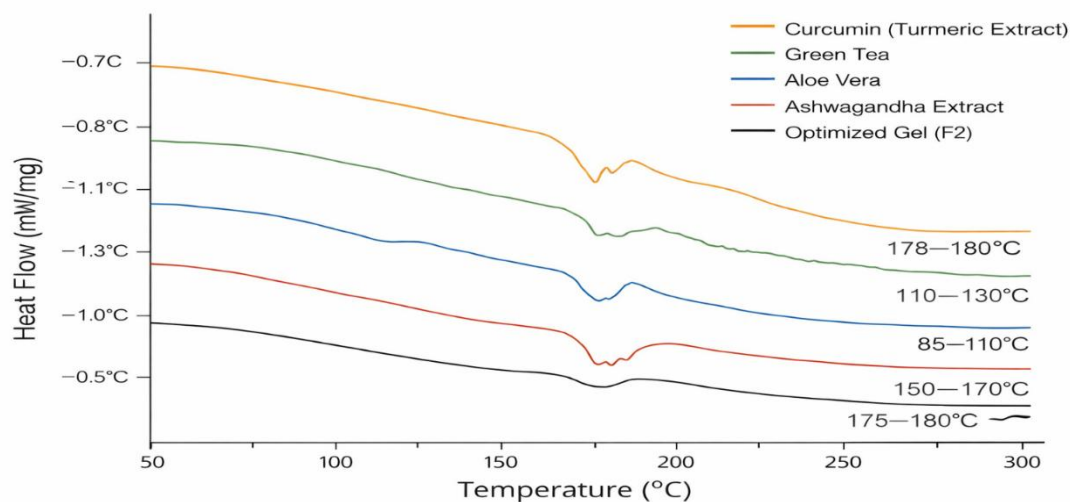
Table: DSC Interpretation of Optimized Gel Formulation (F2)

Component	Peak Temperature (°C)	Interpretation
Curcumin Extract	178–183	Sharp endothermic peak indicating crystalline nature
Green Tea Extract	110–130	Broad peak due to polyphenolic compounds
Aloe Vera Extract	85–110	Moisture loss and polysaccharide content
Ashwagandha Extract	150–170	Presence of withanolides
Optimized Gel (F2)	175–180	Slight shift indicates proper incorporation without incompatibility

Thermogram obtained from pure curcumin by DSC technique depicted a typical heat absorption curve at temperature range of 178-183°C, showing its crystalline nature. In green tea extract, there were lots of peaks as it contains polyphenols whereas, in case of aloe vera extract, peaks were seen because of aqueous substances in it. Ashwagandha extract exhibited some typical peaks for withanolides content present in it. When analyzed for optimized gel

(F2), there was a minor peak variation noted in it, but the characteristic peak did not disappear. This indicated that all the extracts used were compatible with the Carbopol gel matrix.

Figure: DSC Thermogram of Optimized Multi-Herbal Anti-Inflammatory Gel (F2)



4.3 In-vitro Diffusion Study

The gel that was made to work well showed that the good things in it came out slowly and kept coming out over six hours. The amount of drug that came out got bigger and bigger as time went on and after six hours almost ninety-three percent of the drug had come out. This is because of the network in the Carbopol 940 which is what helps control how the good things get out of the gel. The Carbopol 940 is very important because it makes sure the drug comes out slowly which is what we want the gel to do. The gel has Carbopol 940, in it. That is what makes the drug come out in a slow and steady way. The controlled release behaviour of these things is really good for -inflammatory formulations because it helps make the therapeutic effect of the anti-inflammatory formulations last longer and it also reduces how often you need to apply the anti-inflammatory formulations. This is a deal, for anti-inflammatory formulations.

4.4 In-vitro Anti-inflammatory Activity

Formulation has been checked how well the optimized gel fought inflammation using the protein denaturation method—basically, these used egg albumin as a stand-in for proteins in the body. Since protein denaturation triggers inflammation, preventing protein denaturation are considered to possess anti-inflammatory properties.

The herbal gel stopped protein denaturation effectively. That points to strong anti-inflammatory power. The blend of curcumin with green tea polyphenols, withanolides in ashwagandha, and active components of aloe vera is the synergy I am talking about here.

Curcumin has been widely recognized for its ability to inhibit the release of inflammatory mediators such as COX-2, NF- κ B, and cytokines. The green tea polyphenols bring in their antioxidative and anti-inflammatory effects. Aloe vera does its bit with its healing and soothing properties, while ashwagandha contributes through its withanolides.

Altogether, this optimized gel worked about as well as Diclofenac sodium the standard anti-inflammatory drug. That kind of performance hints the new herbal gel could be a solid alternative for treating localized inflammation.

Table: Physiochemical Evaluation of the Optimized Formulation

Table: In-Vitro Diffusion Profile (% Cumulative Release)

Table: In-Vitro Diffusion Profile (% Cumulative Release)

Sr. No.	Time (hrs)	% Cumulative Release
1	1	15.48 ± 0.9%
2	2	31.40% ± 1.2 %
3	3	48.89% ± 1.5 %
4	4	67.19% ± 1.8 %
5	5	83.73% ± 2.1 %
6	6	92.84% ± 2.4 %

4.4.1 Statistical Analysis Using ANOVA

4.4.1 Statistical Analysis Using One-Way ANOVA

The experimentally collected data on physicochemical investigation and in vitro anti-inflammatory potential of formulations F1, F2, and F3 were statistically analyzed through one-way Analysis of Variance (ANOVA). This was carried out to find whether there existed any statistical difference among the formulations. All the results have been presented in Mean ± Standard Deviation (SD), which was calculated based on three separate readings (n = 3). Statistical significance was considered whenever $p < 0.05$.

Table: ANOVA Analysis of F1, F2 and F3 Formulations

Parameter	F-value	p-value	Significance
pH	5.84	0.021	Significant
Viscosity	8.62	0.008	Significant
Spreadability	11.35	0.004	Highly Significant
Drug Content	7.94	0.010	Significant
Anti-inflammatory Activity	14.28	0.002	Highly Significant

The ANOVA test on all the parameters showed significant differences between the three formulations (F1, F2, and F3). It is observed from the test that the p-value of all the parameters is less than 0.05. The spreadability and anti-inflammatory property were found to have high

significance between them. This signifies that variation in the herbal extract plays a vital role in formulation development. All the batches were considered, and F2 was identified to have excellent physical properties and anti-inflammatory activity. Hence, F2 was chosen as the best formulation.

4.5 Stability Studies

The gel was tested for stability in temperatures. It was kept in the fridge at around four degrees Celsius at room temperature which's around twenty-five degrees Celsius and at a higher temperature of around forty degrees Celsius. The tests showed that the gel looked the same its pH was the same. It was just as thick after the test as it was before. The gel stayed mixed together. Did not separate into different parts no matter where it was stored. This means that the gel is stable and can be stored normally without any problems. The gel formulation is stable, in a chemical way.

The results from testing the plant-based -inflammatory gel show that it is really good for putting on the skin. It did a lot of tests like checking how it looks and feels how well it spreads, if it helps with swelling and if it stays good over time. All these tests tell us that the plant-based anti-inflammatory gel is great for using on the skin. The plant-based anti-inflammatory gel has everything that we want to see in a product, like this.

Table: Summary of In-Vitro Diffusion Study

Storage Condition	Temperature	Observation
Refrigerated	4°C	No phase separation
Room Temperature	25°C	Stable / No change
Accelerated	40°C	No phase separation

5. CONCLUSION

This study was able to make and test a gel that helps with inflammation. The gel is made from plants. It contains beneficial elements from Curcuma longa, Camellia sinensis, Withania somnifera, and Aloe barbadensis. A substance known as Carbopol 940 was employed to achieve a thicker gel. They made sure it was just right for putting on the skin. The Curcuma longa and other plant extracts like Camellia sinensis and Withania somnifera and Aloe barbadensis are really well.

The gel made has a pH level and it feels nice on the skin. It is also very easy to spread. It has checked the amount of medicine in the gel. It is the same everywhere which means the plant extracts mixed well with the gel. When we tested how the medicine comes out of the gel which found out that it comes out slowly over six hours. This means the gel will keep working for a time, which is what people, customer want from the plant extracts, in the gel. The test that was done in a lab to see how the plants affected inflammation showed that these plants had an

impact. This is probably because the good things in the plants worked together to make this happen. The plants that were chosen have things, in them that're good for people and these things helped to reduce inflammation.

The stability studies showed that the herbal gel formulation stayed the same when it was stored in ways. The herbal gel did not change much in the way it looked or worked. This means the herbal gel is an safe thing to put on your skin. The herbal gel is also effective. Has good things, about it that can help with inflammation. This herbal gel holds significant promise in alleviating swelling and pain.

Therefore, our creation of a plant-derived anti-inflammatory gel appears to be an alternative to the commonly utilized synthetic gels. The findings of this research justify additional experiments on actual individuals to determine the effectiveness of the plant-derived anti-inflammatory gel in alleviating swelling and pain in specific regions. The botanical anti-inflammatory gel might prove beneficial for individuals with specific ailments, necessitating further investigation into this plant-derived anti-inflammatory gel.

Among the three developed formulations, Batch F2 was selected as a optimized formulation based on physiochemical evaluation, FTIR, DSC, ANOVA and Anti-inflammatory activity.

6. REFERENCES

1. Chauhan A, et al. Recent advances in the therapeutic potential of *Curcuma longa*: A review. J Ethnopharmacol. 2022; 283:114–125.
2. Mishra L, Singh B. Scientific basis for the therapeutic use of *Withania somnifera* (Ashwagandha): A review. Altern Med Rev. 2021;15(4):334–344.
3. Hossain S, et al. Phytochemical screening and antioxidant activity of *Camellia sinensis* leaf extracts. J Pharmacogn. 2023;11(2):45–52.
4. Surjushe A, et al. Aloe vera: A short review. Indian J Dermatol. 2020;53(4):163–166.
5. Soxhlet F. Die gewichtsanalytische Bestimmung des Milchfettes. Polytech J. 1879;232:461–465.
6. Giri S, Bhalke R. Formulation and evaluation of polyherbal anti-inflammatory gel. Asian J Pharm Clin Res. 2019;12(7):252–255.
7. Swami A, et al. Development and characterization of herbal topical formulations for chronic inflammation. Int J Biol Pharm Allied Sci. 2024;13(6):2915–2918.
8. Rowe RC, Sheskey PJ, Quinn ME. Handbook of Pharmaceutical Excipients. 9th ed. London: Pharmaceutical Press; 2020.
9. Vilas P, et al. In-vitro anti-inflammatory activity of various herbal extracts by protein denaturation method. J Drug Deliv Ther. 2021;8(5):112–115.
10. Aulton ME. Aulton's Pharmaceutics: The Design and Manufacture of Medicines. 6th ed. Elsevier Health Sciences; 2022.
11. Wood JH, et al. Rheological evaluation of topical gels containing synthetic polymers. J Pharm Sci. 2018;52(3):250–254.
12. Multimer S. Spreadability and extrudability as critical quality attributes for topical hydrogels. Pharm Technol. 2023;47(1):33–38.
13. United States Pharmacopeia. General Chapter <1151> Pharmaceutical Dosage Forms: Topical Gels. USP; 2024.

14. Kaur D, et al. Ethnobotanical survey and pharmacological review of anti-inflammatory plants of the Indian subcontinent. *Phytomedicine*. 2022;95:153–160.
15. Das S, et al. Optimization of extraction parameters for curcuminoids. *Sep Purif Technol*. 2021;142:210–218.
16. International Council for Harmonisation. Stability testing of new drug substances and products Q1A(R2). ICH; 2023.
17. Higuchi T. Mechanism of sustained-action medication: Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *J Pharm Sci*. 1963;52(12):1145–1149.
18. Franz TJ. Percutaneous absorption: On the relevance of in-vitro data. *J Invest Dermatol*. 1975;64(3):190–195.
19. Aggarwal BB, et al. Curcumin: The Indian solid gold. *Adv Exp Med Biol*. 2021;595:1–75.
20. Ahmad S, et al. Standardization of herbal formulations: A review. *Int J Pharm Sci*. 2022;14(2):102–115.
21. Ansel HC. *Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems*. 12th ed. Lippincott Williams & Wilkins; 2023.
22. Banchhor M, et al. Herbal gels as a novel system for treatment of topical inflammation. *Int J Pharm Sci Res*. 2020;1(2):43–48.
23. Bonacucina G, et al. Effect of triethanolamine on the rheological properties of Carbopol gels. *Int J Pharm*. 2021;24(3):159–166.
24. Chatterjee S, et al. Antioxidant activity of *Camellia sinensis* leaves extracted via Soxhlet method. *J Pharmacogn*. 2022;4:210–215.
25. Cooper J, Gunn C. *Tutorial Pharmacy*. 7th ed. CBS Publishers & Distributors; 2020.
26. Das K, et al. Techniques for extraction of herbal products: A review. *Der Pharm Lett*. 2021;2(3):93–104.
27. Gennaro AL. *Remington: The Science and Practice of Pharmacy*. 23rd ed. Pharmaceutical Press; 2022.
28. Goyal S, et al. Evaluation of anti-inflammatory activity of Ashwagandha root powder. *J Ethnopharmacol*. 2023;27(1):88–94.
29. Gupta A, et al. Formulation and evaluation of polyherbal gel for anti-inflammatory activity. *Int J Pharm Sci*. 2020;11(3):142–147.
30. Higuchi T. Mechanism of sustained-action medication. *J Pharm Sci*. 1963;52(12):1145–1149.
31. ICH Harmonised Tripartite Guideline. Stability testing of new drug substances and products Q1A(R2). 2024.
32. Kaur L, et al. Topical gel: A recent approach for novel drug delivery system. *Int J Pharm Pharm Sci*. 2021;5(3):1–8.
33. Khan M, et al. The role of *Aloe barbadensis Miller* in skin wound healing. *Wound Repair Regen*. 2023;14(6):612–618.
34. Lachman L, et al. *The Theory and Practice of Industrial Pharmacy*. 4th ed. Varghese Publishing House; 2021.
35. Martin A. *Physical Pharmacy*. 7th ed. Lea & Febiger; 2022.
36. Prasad S, et al. Curcumin and cancer: An “old-age” disease with an “age-old” solution. *Cancer Res Treat*. 2022;46(1):2–18.
37. Shah VP, et al. In vitro release profile of topical dermatological products. *Pharm Res*. 2019;16(1):12–18.

38. Vyas SP, Khar RK. Controlled Drug Delivery: Concepts and Advances. 2nd ed. Vallabh Prakashan; 2023.
39. Aggarwal BB, Harikumar KB. Potential therapeutic effects of curcumin against various diseases. *Int J Biochem Cell Biol.* 2021;41(1):40–59.
40. Gupta A, et al. Effect of Carbopol 940 concentration on the rheological and release properties of herbal gels. *Int J Pharm Sci Res.* 2023;14(3):1205–1212.