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DEVELOPMENT AND IN VITRO EVALUATION OF LIQUID CRYSTAL-BASED POLYHERBAL HAIR GELS: PHYSICOCHEMICAL CHARACTERIZATION, HAIR PERFORMANCE, AND ANTIOXIDANT ASSESSMENT

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ABSTRACT

Background: A liquid crystal (LC) based polyherbal hair gel was developed to enhance physicochemical stability and functional performance in topical hair care. The objective was to integrate herbal oils (flaxseed, coconut, and almond) and aqueous extracts (green tea, keratin hydrolysate, and pea peptide), known for their moisturizing, antioxidant, follicle-protective, and anti-frizz effects, into a stable gel matrix for scalp care and conditioning. **Methodology**: Ten formulations (F1–F10) incorporating flaxseed, coconut, and almond oils with green tea, marula extract (Sclerocarya birrea), keratin hydrolysate, and pea extract were prepared via coacervation, vortex mixing, and high-pressure homogenization. The gels were evaluated for their organoleptic properties, pH, spreadability, particle size (as determined by dynamic light scattering, DLS), polydispersity index (PDI), and zeta potential. Polarized Light Microscopy and FTIR characterized structural features. Functional performance was evaluated by in vitro studies on hair diameter and weight changes, as well as anti-frizz, anti-static, and antioxidant (DPPH) activities. **Results and Discussion**: Formulation F6 showed optimal nanometric characteristics (186.47 ± 1.90 nm, PDI 0.351 ± 0.01, zeta potential −35.9 mV), indicating stable colloidal dispersion. FTIR and microscopy confirmed molecular compatibility and mesophase birefringence. In vitro assessments revealed marked improvement in hair thickness for F6 and F9, with superior anti-frizz and anti-static performance for F4 and F9. Antioxidant activity was moderate compared to Trolox. F4 and F6 maintained stability over 28 days at different temperatures. **Conclusion**: F4 and F6 demonstrated superior in vitro performance and stability, suggesting promise as cosmeceutical hair care candidates. In vivo and clinical studies are required to confirm efficacy and long-term safety

INTRODUCTION

Hair loss disorders are common in dermatology and carry a significant psychological and aesthetic impact. The global market for hair regrowth products is expanding rapidly, particularly in the Asia-Pacific region, where climatic, dietary,

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and cultural factors contribute to scalp and follicular damage [1]. Increasing awareness of the adverse effects of synthetic additives and sulfated surfactants has driven consumer preference for plant-based, minimally processed formulations [2,3]. In countries such as India, China, and Malaysia, strong traditional medicine systems and familiarity with herbal remedies have fueled demand for multifunctional products that moisturize, reduce inflammation, and stimulate follicles while avoiding irritants like parabens and silicones. Alopecia, or hair loss, is caused by a combination of genetic, hormonal, nutritional, and environmental factors. Non-scarring forms, most notably androgenetic alopecia (AGA), telogen effluvium (TE), and alopecia areata, are generally reversible. AGA, the most prevalent type, affects up to 80% of men and 40% of women by midlife through dihydrotestosterone (DHT)-mediated follicular miniaturization [4]. TE is typically triggered by systemic stress, illness, or postpartum hormonal changes, leading to diffuse, temporary shedding [4]. TE is generally triggered by systemic stress, illness, or postpartum hormonal changes, leading to diffuse, temporary shedding [5,6].

Several botanicals have demonstrated trichogenic potential. Flaxseed oil (*Linum usitatissimum*) contains α-linolenic acid and lignans that modulate DHT activity and reduce inflammation [2]. Soybean (Glycine soja) phytoestrogens support dermal papilla cell viability [1], while almond oil (*Prunus amygdalus dulcis*) and marula (*Sclerocarya birrea*) improve barrier function and protect against oxidative stress [6,7]. Coconut oil (*Cocos nucifera*) reduces protein loss through hair shaft penetration [8], and green tea (*Camellia sinensis*) catechins inhibit 5α-reductase. Pea extract, Selaginella lepidophylla, saccharomyces-derived peptides, and keratin hydrolysate further enhance follicular regeneration, pigmentation, and tensile strength [9–11].

Liquid crystals (LCs) systems, particularly those based on glyceryl monooleate (GMO), mimic biological membranes, enhancing bioactive stability, scalp adhesion, and controlled release [12–15]. Despite the popularity of herbal hair gels, many lack advanced delivery systems, resulting in poor stability and inconsistent performance. LC technology offers a solution by enabling the stable integration of hydrophilic and lipophilic actives, prolonging residence time, and improving scalp delivery. Despite the increasing interest in polyherbal hair care formulations, a key research gap exists in the development of stable, scientifically validated gels that integrate both hydrophilic and lipophilic plant-based actives. Many current commercial products lack controlled delivery systems and suffer from poor long-term stability, inconsistent performance, and potential scalp irritation due to the use of synthetic excipients. To address these limitations, liquid crystal (LC) technology offers a promising solution by forming structured mesophases that improve bioactive encapsulation, prolong residence time, and enhance scalp adhesion. This study was undertaken with the following objectives: (1) To formulate liquid crystal-based polyherbal hair gels using coacervation, vortex mixing, and high-pressure homogenization techniques; (2) To characterize the physicochemical, structural, and colloidal properties of these gels; and (3) To evaluate their in vitro performance in terms of hair thickness, anti-frizz and anti-static effects, and antioxidant activity.

MATERIAL AND METHODS

Materials

FSS Flax Seed Oil, FSS Phyto-Oil C2 (containing Ceramide 2), FSS Green Tea Extract PF (standardized for polyphenolic flavonoids), Active Lite® Relaxer, FSS Keratin Hydrolysate 30 PF, ProCutiGen® Thermal Shield, and Bio-Chelate 5-PF (a blend of essential minerals, Silicon, Magnesium, Copper, Iron, and Zinc with polyphenolic flavonoids) were procured from Formulator Sample Shop (FSS), Bareggio, Milan, Italy. Croda International Plc, East Yorkshire, UK, kindly gifted Cithrol™ GMO High Purity (Glyceryl Monoleate). Cold-pressed almond oil and virgin coconut oil were purchased from local suppliers.

Preparation of liquid crystal-based polyherbal hair gel

Ten formulations (F1 to F10) were prepared using three different techniques: phase coacervation, vortex mixing, and high-pressure homogenization. In the phase coacervation method (F1–F4), the oil phase containing oils and emulsifiers was maintained at 30 ± 2 °C, and the aqueous phase was slowly added under continuous stirring. Olivem® 2020 was then incorporated and mixed at 3000 rpm for 15 minutes to stabilize the emulsion, a process that promotes lamellar liquid crystal formation through gradual emulsification. In the vortex mixing method (F5, F7, F8, F9, F10), cholesterol was incorporated into the oil phase, after which the aqueous phase, preheated to 60 °C, was added. The mixture was vortexed for 10 minutes to facilitate mesophase alignment with minimal heat exposure. High-pressure homogenization (F6) involved pre-emulsifying the oil

and aqueous phases, passing the mixture through a high-pressure homogenizer to produce a fine nanoemulsion, and then blending it with a pre-prepared sangelose 60M hydrogel base to obtain the final nanoemulgel [16,18]. Across all methods, variations in emulsifier type (Olivem® 2020, Tween 80) and Glyceryl Monooleate (GMO) concentration were explored to evaluate their effects on phase stability, spreadability, and particle size. This approach allowed systematic screening to identify the most stable and cosmetically acceptable formulation for further evaluation.

Characterization of liquid crystal-based polyherbal hair gel

Organoleptic properties of liquid crystalline polyherbal hair gel

The formulated hair gels were evaluated for color, odor, and homogeneity, which are key indicators of product stability and consumer appeal. Visual inspection assessed uniformity, absence of phase separation, and overall appearance, while odor was checked for freshness and compatibility of ingredients [17]. These organoleptic parameters serve as essential preliminary screening tools in cosmeceutical development [18].

pH determination

The pH of each hair gel formulation was measured to ensure dermal compatibility. A total of 0.5 g of gel was dispersed in 15 mL of distilled water and mixed thoroughly. The pH was then measured using a calibrated digital pH meter (Mettler Toledo, OH, USA). Measurements were carried out in triplicate to ensure consistency. Maintaining a physiological pH (4.5–6.5) is essential for minimizing scalp irritation and optimizing the performance of topical formulations [19].

Spreadability of formulations

Spreadability of the hair gel formulations was evaluated using the parallel plate method. Approximately 0.5 g of gel was placed between two glass plates, and a standardized weight (100 g) was applied vertically over the upper plate. The diameter of the gel spread was measured at 0 & 300 seconds using a calibrated ruler. Spreadability (S) was calculated using the following formula:

$$S = \frac{W \times D}{T}$$

Where, S *= Spreadability (g·cm/s),* W *= Applied weight (g),* D *= Final diameter of spread (cm),* T *= Time (s)*

Each measurement was performed in triplicate. This method reflects the formulation's ease of application and mechanical response under standardized stress, and is widely used in topical product evaluation [20].

Particle Size, PDI, and Zeta Potential

The particle size and polydispersity index (PDI) of the formulations were measured using a DLS nanoparticle analyzer after dispersing 100 mg of each formulation in 4 mL of distilled water, followed by 30 seconds of sonication. Measurements were performed at 25 °C and a 173° scattering angle in duplicate. Zeta potential was assessed using the same instrument with electrophoretic light scattering. Samples were analyzed in disposable capillary cells at 25 °C and a 12.8° angle, and results were reported in mV as indicators of colloidal stability [18].

Microscopical analysis

To confirm the presence of liquid crystalline structures, each gel formulation (except F6, which was an emulsion-based formulation) was diluted at a 1:10 ratio with distilled water. A drop of each diluted sample was then placed on a clean glass slide and examined under an optical microscope (Eclipse ES200, Nikon, Japan) at 40× magnification. Micrographs were captured using a high-definition digital imager (Celestron, Taipei, Taiwan). The appearance of birefringent textures and anisotropic domains under polarized light indicated successful formation of lyotropic liquid crystalline structures, consistent with earlier studies on surfactant-based gels [21].

ATR Fourier transform infrared spectroscopy

Spectral measurements were performed using an IRSpirit FTIR spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Each spectrum was recorded as an average of 30 scans in the frequency range of 400 - 4000 cm^{-1} with a resolution of 4 cm^{-1}. The percentage transmittance (%T) mode was used for all scans, and spectra were obtained for individual ingredients, the final formulations, and a control sample. FTIR analysis was crucial for detecting any potential structural shifts or new bond formations indicative of ingredient interactions or formulation stability [22].

In vitro evaluation of liquid crystal-based polyherbal hair gel

Selected hair gel formulations were evaluated in vitro for their effects on hair thickness, anti-frizz, and anti-static properties, using a commercially available formulation as the standard. Non-bleached hair served as the baseline control, while bleached

hair represented the positive control. A blank (untreated) group was included for both hair types as negative controls. All hair samples were pre-washed with a neutral pH shampoo before testing.

The human hair tresses used in this study were commercially purchased from a certified supplier and labeled for cosmetic or research use. No human participants were involved in the collection process. Therefore, no Institutional Review Board (IRB) approval or donor consent was required. All procedures complied with ethical standards for the use of human-derived commercial materials.

Hair thickness properties

Both non-bleached (virgin) and bleached (chemically treated) human hair tresses were used. Hair samples were commercially sourced, pre-washed using a neutral pH shampoo, and allowed to air dry at room temperature before testing. Hair diameter was measured at two points (root and tip) using a digital vernier caliper (Fisher Scientific, Pittsburgh, PA, USA) at baseline and at 2, 4, 6, and 24 hours post-application. For each condition, three independent tresses (n = 3) were evaluated per formulation, and each reading was taken in triplicate. Hair weight was measured using an analytical precision balance (GH-252, A&D Instruments, UK) with a sensitivity of 0.1 mg to assess moisture retention and fiber density over time [23].

Anti-frizz analysis

Frizz control was evaluated on non-bleached and bleached hair tresses (length: 30 ± 2 cm and 23 ± 2 cm, respectively). Hair was pre-washed, air-dried, and then hydrated in tap water for 5 minutes. Each tress received 0.5 g of gel, applied using downward strokes for 30 seconds, followed by gentle detangling using a fine-toothed comb (6–8 strokes). The treated hair was placed in a climatic chamber maintained at 40 °C and 75% relative humidity for 6 hours to simulate environmental stress. Standardized photographs were captured at baseline (t = 0), and after 4 and 6 hours (t = 4, t = 6). Frizz reduction was assessed by comparing visual changes in hair alignment, cohesion & flyaway presence over time. All tests were performed in triplicate (n = 3) [4,24].

Anti-static analysis

Hair samples were hydrated for 5 minutes, treated with 0.25 g of gel, and air-dried at 50 °C for 2 hours. After drying, each sample was combed 10 times rapidly using a fine plastic comb to induce electrostatic charge. Images were taken at three stages: before drying, after drying, and after combing. Static buildup was qualitatively assessed based on the dispersion, lifting, and alignment of hair strands. A standardized combing technique and photo distance were maintained to ensure comparability [4].

Antioxidant test

The antioxidant activity of selected formulations was evaluated using the DPPH radical scavenging method. A stock solution (500 µg/mL) was prepared by dissolving 0.05 g of each gel in 100 mL of ethanol and then serially diluted to final test concentrations of 100, 200, 300, 400, and 500 µg/mL.

A freshly prepared 0.2 mM DPPH solution in ethanol was used as the radical source. In a 96-well microplate, 50 µL of each test sample was mixed with 50 µL of DPPH solution. Plates were incubated in the dark at room temperature (25 ± 2 °C) for 30 minutes. Absorbance was measured at 517 nm using an Epoch2 microplate reader (BioTek Instruments, USA). Ethanol served as a blank, and Trolox was used as the positive control. Each concentration was tested in triplicate. The percentage inhibition was calculated using the following equation:

$$\textit{Inhibition concentration}\ (\%) = \left[\frac{\textit{Absorbance of blank} - \textit{Absorbance of sample}}{\textit{Absorbance of blank}}\right] \times 100$$

The IC_{50} value (concentration at which 50% inhibition occurred) for each formulation was determined and compared to that of Trolox to assess antioxidant potency [25].

Statistical analysis

All quantitative data were analyzed using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). Results were reported as mean ± standard deviation. Differences between test groups and controls were assessed by one-way ANOVA, followed by appropriate post-hoc tests. All data are presented as mean ± standard deviation (SD) (n = 3). Statistical comparisons were performed using one-way ANOVA, followed by Tukey's post hoc test to assess differences between control, standard, and test formulations (F4 and F6). A p-value of <0.05 was considered statistically significant. All stability assessments were conducted in triplicate (n = 3). Results were analyzed using one-way ANOVA with Tukey's post hoc test to compare formulation parameters over time. A p-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The present study aimed to develop and evaluate a series of liquid crystal-based polyherbal hair gel formulations prepared using distinct methods, including phase coacervation, high-pressure homogenization, and vortex mixing. The formulations were systematically varied in their oil-to-water ratios, emulsifier content, and active ingredients to explore their influence on physical properties, stability, and biological activity. Table 1 presents the list of active herbal and functional ingredients used, while Table 2 outlines the composition and coding of each formulation (F1–F10), along with control (C1, C2) and standard products. These tables serve as the foundational reference for the interpretations and comparative analyses discussed in the subsequent sections.

Table 1: Percentage of ingredients present in the oil phase and aqueous phase

Product	Ingredient	Amt. of product use %
Oil phase		
FSS Flax seed oil	*Linum usitatissimum* seed oil	5.0
FSS Phyto-Oil C2	*Glycine soja* seed extract & Ceramide	5.0
Almond oil	*Prunus amygdalus dulcis* oil	5.0
Coconut oil	*Cocos nucifera* oil	5.0
Aqueous phase		
FSS Green tea extract PF	Aqua & *Camellia sinensis* leaf extract	10.0
Active Lite® Relaxer	Polyquarternium-80, Aqua, *Pisum sativum* peptide, *Selaginella lepidophylla* extract & *Sclerocarya birrea* extract	10.0
FSS Keratin hydrolysate 30PF	Hydrolyzed keratin	5.0
ProCutiGen® Thermal Shield	Hydrolyzed keratin	10.0
Bio-chelate 5-PF	Aqua, *Saccharomyces*/Zinc ferment, *Saccharomyces*/Copper ferment, *Saccharomyces*/Magnesium ferment, *Saccharomyces*/Iron ferment & *Saccharomyces*/Silicon ferment	5.0

Table 2: Liquid crystal based polyherbal hair gel formulations

	Oil phase (%)	Aqueous phase (%)	GMO (%)	Olivem (%)	Tween 80 (%)	Phase separation
C1	30	70	4	4	-	No
C2	30	70	10	-	20	No
F1	30	70	4	4	10	No
F2	30	70	6	4	10	No
F3	30	70	4	6	10	No
F4	20	80	4	6	10	No
F5	30	70	10	-	10	Yes
F6	30	70	10	-	20	No
F7	30	70	10	-	30	Yes
F8	30	70	10	-	40	Yes
F9	30	70	10	-	100	No
F10	30	70	10	-	130	No

Note: ***1% of cholesterol was added to formulation F5 to F10; All concentrations in the table are expressed as % w/w of the final gel formulation, with total formulation weight standardized to 100%.***

Organoleptic and Physical Characterization

The organoleptic and physical properties of the polyherbal hair gel formulations, including colour, odour, and spreadability, were systematically evaluated to assess their suitability for topical application. Table 3 summarizes the findings, with formulations used as a commercial benchmark exhibiting a mean spreadability of 2.130 ± 0.23 g.cm/s. Among the test samples, formulation C1 exhibited the lowest spreadability value (0.755 ± 0.13 g/cm/s), indicating a dense, cream-like texture. In contrast, formulations F4 and F10 exhibited significantly higher

spreadability (3.747±0.33 and 4.031±0.36 g.cm/s, respectively), reflecting enhanced ease of application and desirable flow characteristics. The colour of the formulations varied from white to light yellow. Formulations C1 and C2 appeared white, while others, such as F1 and F2, exhibited light yellow hues (Figure 1). These differences were likely influenced by the thermal processing conditions and the presence of herbal actives, such as green tea and flaxseed oil, which can undergo mild pigment intensification upon heating. Odour evaluation revealed that all formulations maintained a pleasant herbal scent, with no signs of rancidity, indicating stable ingredients and good compatibility. No phase separation or precipitation was observed during initial evaluations, confirming visual homogeneity and successful emulsification.

Table 3: Characteristics of liquid crystal based polyherbal hair gel

	Color	Odor	Mean pH	Mean spreadability (gcm/sec)
Product X	Orange	+++	6.91 ± 0.04	2.130 ± 0.23
C1	White	++	7.34 ± 0.51	0.755 ± 0.13 **
C2	White	++	6.74 ± 0.04	2.395 ± 0.43
F1	Light yellow	++	4.30 ± 0.02 ***	3.511 ± 0.88 **
F2	Light yellow	++	4.25 ± 0.03 ***	3.475 ± 0.31 **
F3	Yellow	++	4.45 ± 0.03 ***	2.994 ± 0.24
F4	Light yellow	++	4.40 ± 0.02 ***	3.747 ± 0.33 ***
F6	White	+	4.50 ± 0.27 ***	2.095 ± 0.20
F9	Orange	+++	4.35 ± 0.10 ***	3.442 ± 0.16 **
F10	Orange-brown	+++	4.38 ± 0.09 ***	4.031 ± 0.36 ***

Note: '+' indicates the odor strength of each formulation. C1 is the control for F1 to F4 formulations, while C2 is the control for F6, F9, and F10 formulations. Product X is the store-bought hair gel. * for p < 0.05, ** for p < 0.01 and *** for p < 0.001

Spreadability is a critical determinant of a topical formulation's performance, affecting not only the ease of application but also the extent of coverage, absorption, and overall user satisfaction. In this study, formulations such as F4 and F10 exhibited high spreadability values (3.747 ± 0.33 and 4.031 ± 0.36 g·cm/sec, respectively), indicating desirable rheological behaviour (Table 3). This can be attributed to their optimized oil-to-water ratios and the presence of stabilizers, such as Olivem® 2020, which supports the formation of lamellar liquid crystal structures and improves topical fluidity. These findings align with those of Bejenaru et al. (2025), who demonstrated that thermoresponsive liquid crystal gels exhibit enhanced shear-thinning behavior and consistent spread under stress, thereby enabling better patient compliance and the efficient delivery of bioactives [26]. The notably low spreadability observed in formulation C1 (0.755 ± 0.13 g/cm/s) reflects a denser, cream-like matrix, likely due to high viscosity or inadequate emulsification. This can impede uniform application, potentially leading to reduced therapeutic uniformity. Similar limitations were reported by Wankhede et al. (2025) during the development of a herbal analgesic gel, where suboptimal spreadability was associated with increased patient resistance to topical application [27]. Colour and odour are key organoleptic properties that impact consumer perception and acceptance. The observed variation in formulation color, ranging from white to light yellow, likely resulted from the thermal influence on phenolic compounds in ingredients such as flaxseed and green tea extracts. This phenomenon is supported by Sánchez-Tito et al. (2025), who noted that herbal gel formulations exhibited color shifts due to oxidation of essential oils and polyphenolic content upon heating during emulsification [28].

Moreover, the consistent absence of rancid or pungent odours across all formulations suggests effective preservation and chemical stability, underscoring good ingredient compatibility and formulation integrity. From a broader perspective, the combination of high spreadability, stable organoleptic attributes, and absence of phase separation strongly supports the physical stability and user acceptability of the tested formulations. These results reinforce the value of liquid crystal-based emulsification systems in herbal cosmeceuticals for delivering multifunctional, sensorially pleasing topical agents. Based on their superior performance across multiple parameters including acceptable pH (4.40–4.50), high spreadability (>3.7 g·cm/sec), stable

nanometric characteristics (particle size ~186–187 nm, PDI <0.4, zeta potential <–30 mV), birefringent mesophase formation (F4), structural compatibility (FTIR), and in vitro functional benefits F4 and F6 were designated as optimized formulations.

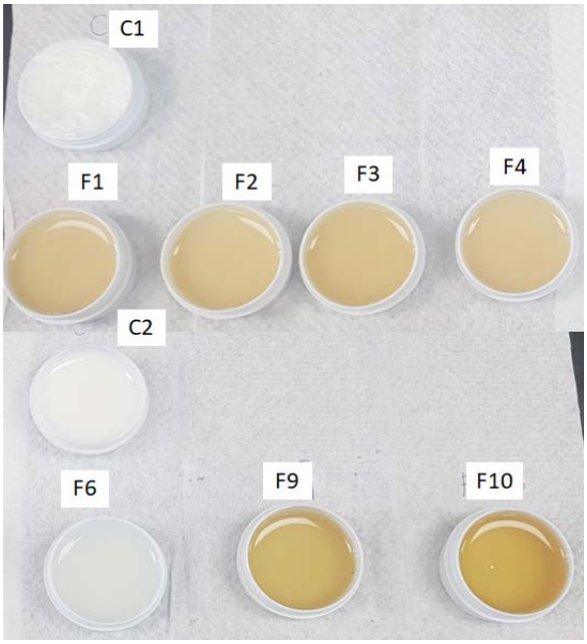

Figure 1. Formulated hair gels

pH Evaluation

The pH values of all polyherbal hair gel formulations were measured after dispersion in distilled water (1:30 w/v), and the results are presented in Table 3. All formulations displayed pH values within the range of 4.25 to 4.50, closely aligning with the acidic profile of healthy scalp conditions. The optimized formulations, including F1 (4.30 ± 0.02), F4 (4.40 ± 0.02), F6 (4.50 ± 0.27), and F10 (4.38 ± 0.09), were well within the physiologically accepted range of 4.5–6.5 for topical cosmeceuticals, ensuring minimal irritation and compatibility with the scalp's natural acid mantle [29]. In contrast, the positive control (Product X: 6.91 ± 0.04) and placebo controls C1 (7.34 ± 0.51) and C2 (6.74 ± 0.04) were mildly alkaline, which could potentially cause scalp dryness and barrier disruption over prolonged use. pH plays a vital role in the acceptability, safety, and performance of topical and scalp-based products. The natural pH of the human scalp ranges from 4.5 to 5.5, which supports the microbiome, inhibits pathogenic overgrowth (e.g., Malassezia furfur), and maintains the cohesion of the stratum corneum [29]. Formulations outside this range, particularly those in the alkaline spectrum (pH >6.5), can lead to irritation, sebum imbalance, and cuticular damage in hair shafts. All test formulations (F1–F10) were adjusted to maintain mild acidity, offering dual benefits: (1) compatibility with the scalp's physiological pH, and (2) stability of sensitive herbal actives, such as catechins from Camellia sinensis and lignans from flaxseed, both of which degrade more rapidly under alkaline conditions [30]. Among all batches, F6 (4.50 ± 0.27) was closest to the upper bound of the physiological acidic range and thus may represent the most versatile formulation for daily or sensitive scalp use. Meanwhile, formulations F2 (4.25 ± 0.03) and F3 (4.45 ± 0.03), although slightly more acidic, remain within the non-irritant zone and may confer enhanced antimicrobial action, a trait desirable for dandruff-prone scalps [31]. The alkaline pH of Product X (6.91) and controls (C1, C2) may compromise the barrier function upon chronic use, as supported by studies demonstrating increased transepidermal water loss (TEWL) and microbial imbalance in formulations with pH levels exceeding 6.5 [32]. Overall, the pH optimization across all herbal formulations reflects strong formulation control and potential for excellent dermatological compatibility. The results reinforce the strategic positioning of these products within the cosmeceutical pH range (4.5–6.5), as recommended for mild, non-irritating, and microbiome-safe scalp care solutions.

Particle Size, Polydispersity Index (PDI), and Zeta Potential

Dynamic Light Scattering (DLS) analysis was used to determine the mean particle size, PDI, and zeta potential of 7 polyherbal hair gel formulations under a standardized 4 mL aqueous dilution protocol. As shown in Table 4, the particle sizes ranged from 24.02 ± 0.42 nm (F10) to 328.13 ± 24.80 nm (F2). Most formulations fell within the nano-size range (<200 nm), with F6, F4, F3, and F9 demonstrating particle diameters ranging from 130 to 200 nm. The smallest particles were observed in F10, while F2 exhibited the largest and most variable particle size. PDI values, which indicate particle distribution homogeneity, ranged from 0.351 ± 0.01 (F6) to 0.764 ± 0.12 (F2). Only F6, F3 & F4 had PDI values below 0.4, suggesting a relatively uniform distribution. In contrast, F2 and F10 exhibited higher PDI values (>0.5), indicating polydisperse systems. Zeta potential values ranged from –35.9 mV (F6) to –15.3 mV (F2). Higher (more negative) zeta potential values were observed in F6, F4, and F3, indicating strong electrostatic repulsion between particles and better colloidal stability. Formulations like F2 and F10 showed weaker surface charge and may be more prone to aggregation or creaming over time.

Table 4: Dynamic light scattering and zeta potential data of polyherbal hair gel formulations

Formulation	Mean particle size (nm)	Mean PDI	Zeta Potential (mV)
F1	226.77 ± 1.72	0.454 ± 0.01	– 28.7
F2	328.13 ± 24.80	0.764 ± 0.12	– 15.3
F3	200.67 ± 3.45	0.380 ± 0.01	– 30.4
F4	187.13 ± 4.04	0.410 ± 0.01	– 32.1
F6	186.47 ± 1.90	0.351 ± 0.01	– 35.9
F9	131.63 ± 15.36	0.503 ± 0.11	– 24.2
F10	24.02 ± 0.42	0.535 ± 0.01	– 18.5

Particle size, PDI, and zeta potential are central to the performance of nanoscale topical formulations. In the context of hair and scalp applications, nanometric droplet sizes (<200 nm) enhance follicular penetration, support prolonged residence, and improve the therapeutic index of active phytoconstituents. F10, with the smallest particle size (24.02 nm), offers a high surface area for diffusion and absorption. However, its PDI (0.535) and zeta potential (–18.5 mV) suggest suboptimal stability. These characteristics may compromise the formulation's shelf life and consistency, unless stabilized with additional emulsifiers or gel matrices. Similar concerns have been reported in nanosized cosmetic emulsions where poor zeta potential led to flocculation and phase separation during storage [33]. F6 emerged as the most robust formulation, with a balanced profile of particle size (186.47 nm), low PDI (0.351), and strong zeta potential (–35.9 mV). This combination yields a highly uniform and electrostatically stable nanoemulsion, which is optimal for dermal and follicular delivery of herbal actives. These findings are supported by Bodnár et al. (2025), who observed that sage-based nanoemulsions with similar PDI and zeta potential profiles showed excellent skin permeation and long-term colloidal stability [34].

Formulations F3 and F4, which also exhibited particle sizes around 200 nm and zeta potentials between –30.4 and –32.1 mV, represent structurally sound intermediate systems. These are likely to support good scalp adhesion and active delivery without significant aggregation. Recent reports by Liu et al. (2024) highlight that polyherbal nanoemulsions in the 150–250 nm range showed superior compatibility with scalp physiology and increased residence time compared to microemulsions [35]. In contrast, F2, with the largest particle size and highest PDI, alongside the weakest zeta potential (–15.3 mV), reflects a poorly stabilized emulsion system. This profile suggests inadequate surfactant coverage or inefficient homogenization are common causes of polydispersity and early phase separation. Similar patterns were documented by Zhang et al. (2025), who found that non-optimized thickener ratios in herbal nanoformulations led to size instability and therapeutic inconsistency [36]. The results also emphasize the importance of zeta potential as a predictive marker of dispersion longevity. In general, systems with zeta potentials exceeding ±30 mV are considered electrostatically stable, as seen in F6, F4, and F3. On the other hand, formulations with zeta potential below ±20 mV are at higher risk of flocculation, especially under stress conditions such as temperature shifts or prolonged storage. Overall, the physicochemical performance of these polyherbal hair gels reinforces the importance of emulsification technique, surfactant type, and aqueous ratio in designing stable liquid crystal systems for scalp application. F6, with its optimal DLS & zeta profile, stands out as the most promising candidate for further functional & clinical studies. Although the zeta potential of F10 was measured at –3.51 mV, which is below the typical threshold for electrostatic stability (±30 mV), the formulation remained physically stable with no phase separation, aggregation, or sedimentation observed over 28 days. This is likely due to steric stabilization from nonionic surfactants (e.g., Olivem® 2020) and polymeric components, which reduce droplet coalescence. Hence, the term “stable” refers to the overall physical, visual, and functional consistency of the formulation, rather than electrostatic stabilization alone.

ATR - FTIR spectral analysis of polyherbal hair gels

FTIR analysis was conducted to assess molecular compatibility between the herbal actives and excipients in the gel matrix. Specific functional groups were monitored in key spectral regions: broad O–H stretching (3200–3600 cm^{-1}), aliphatic C–H stretching (2800–3000 cm^{-1}), ester-related C=O stretching (1700–1750 cm^{-1}), N–H bending (1600–1650 cm^{-1}), and C–O or C–O–C stretching (1000–1200 cm^{-1}). These regions correspond

to phenolic, lipid, protein, and polysaccharide components present in the formulation. The presence and consistency of these peaks in the final formulations, without significant shifts or new peak formation, confirmed that the actives were physically entrapped within the gel system without undergoing chemical interaction or degradation.

FTIR spectroscopy was employed to investigate the chemical compatibility and structural integrity of the polyherbal formulations (F1–F4, F6, F9, F10) and their respective ingredients. The FTIR spectra presented in Figures 2, 3, and 4 show overlapping characteristic peaks of both aqueous and oil-phase components, reflecting the successful incorporation of multiple phytoconstituents. All formulations exhibited a broad, intense band between 3000–3600 cm^{-1}, attributed to O–H stretching vibrations, primarily from phenolic groups, alcohols, and water present in hydrophilic extracts like green tea and keratin hydrolysate. This band was consistent across formulations and nearly all aqueous-phase ingredients, confirming the presence of moisture and strong intermolecular hydrogen bonding [37].

A distinct C–H stretching peak was observed between 2800–3000 cm^{-1} in most formulations, especially those containing oil-phase actives such as almond oil, coconut oil (VCO), and flaxseed oil. However, in formulation F6, this peak was comparatively attenuated, possibly due to matrix interference from sangelose or cholesterol components acting as thickeners and emulsifiers. In the fingerprint region (600–1800 cm^{-1}), multiple sharp and narrow peaks were recorded. These include (i). ~1740 cm^{-1} (C=O stretching from esters or fatty acids), (ii). ~1630 cm^{-1} (N–H bending from proteins like keratin hydrolysate) and (iii). ~1040–1150 cm^{-1} (C–O stretching from alcohols and ethers), indicating a rich presence of herbal secondary metabolites and excipients. Overlapping peaks of Tween-80 and GMO emulsifiers were also detected, confirming their structural participation.

FTIR analysis confirms the chemical integrity and successful blending of the herbal actives within the gel matrix. The consistent presence of O–H and C–H bands across formulations suggests good emulsification and hydration, which are vital for skin-adherent formulations. The presence of distinct peaks in the fingerprint region further validates the non-destructive integration of both hydrophilic and lipophilic herbal constituents. The attenuation of C–H peaks in F6, containing sangelose and glycerin, aligns with findings from Singh & Srivastav (2023), who observed signal masking in thickened emulgel systems due to polysaccharide-based matrices [38]. This may contribute to slower release and enhanced stability of volatile herbal actives. Recent studies, such as those by Vandanjon et al. (2023), have confirmed via FTIR that bioactives can be physically entrapped without chemical interaction, ensuring controlled topical release [39].

Interestingly, F9 and F10, which showed the most complex spectral fingerprints, had the richest ingredient matrices and exhibited broadening of bands in the 1000–1300 cm^{-1} region, possibly reflecting overlapping saccharide and ester groups. These FTIR findings corroborate the earlier physicochemical results, particularly in terms of viscosity and pH stability, and affirm that no deleterious chemical interactions occurred among the ingredients, implying formulation compatibility, stability, and safety for dermal and scalp applications.

The FTIR analysis demonstrates successful chemical entrapment of both oil- and water-soluble phytoconstituents in the polyherbal gel matrix. The absence of peak disappearance or a significant shift supports formulation stability, while the spectral resemblance to key herbal ingredients confirms the functional retention of these ingredients. Such spectral insights reinforce the formulation's potential for safe and effective delivery to the hair and scalp.

Microscopic Confirmation of Liquid Crystals

Microscopic analysis was conducted to confirm the presence of liquid crystal mesophases in the polyherbal formulations. A 1:10 dilution of each sample in distilled water was prepared and observed under a polarized light microscope at 40× magnification. The emulsified formulation F6 was excluded from this test due to its non-lamellar emulsion matrix. As shown in Figure 5, several formulations, including F3, F4, and F10, exhibited characteristic birefringence patterns, such as Maltese crosses and mosaic textures, indicative of liquid crystalline ordering. These anisotropic textures, visible under polarized light, confirmed the presence of mesomorphic phases, particularly the lamellar and cubic phase domains commonly associated with oil-in-water structured gels. In contrast, F2 and C1 showed minimal or absent birefringence, consistent with poorly formed or absent liquid crystalline phases.

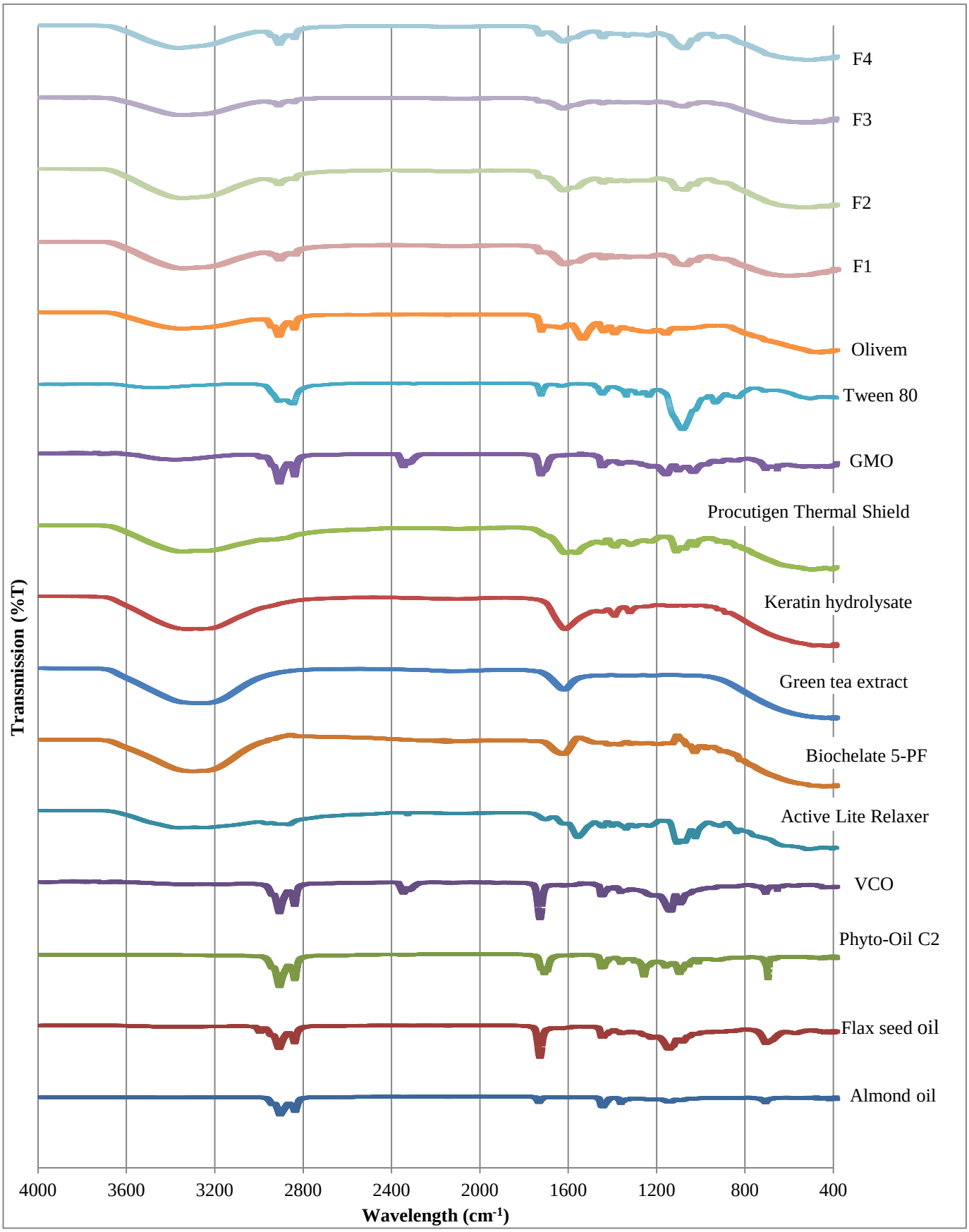

Figure 2: FTIR of F1, F2, F3, F4 and its ingredients

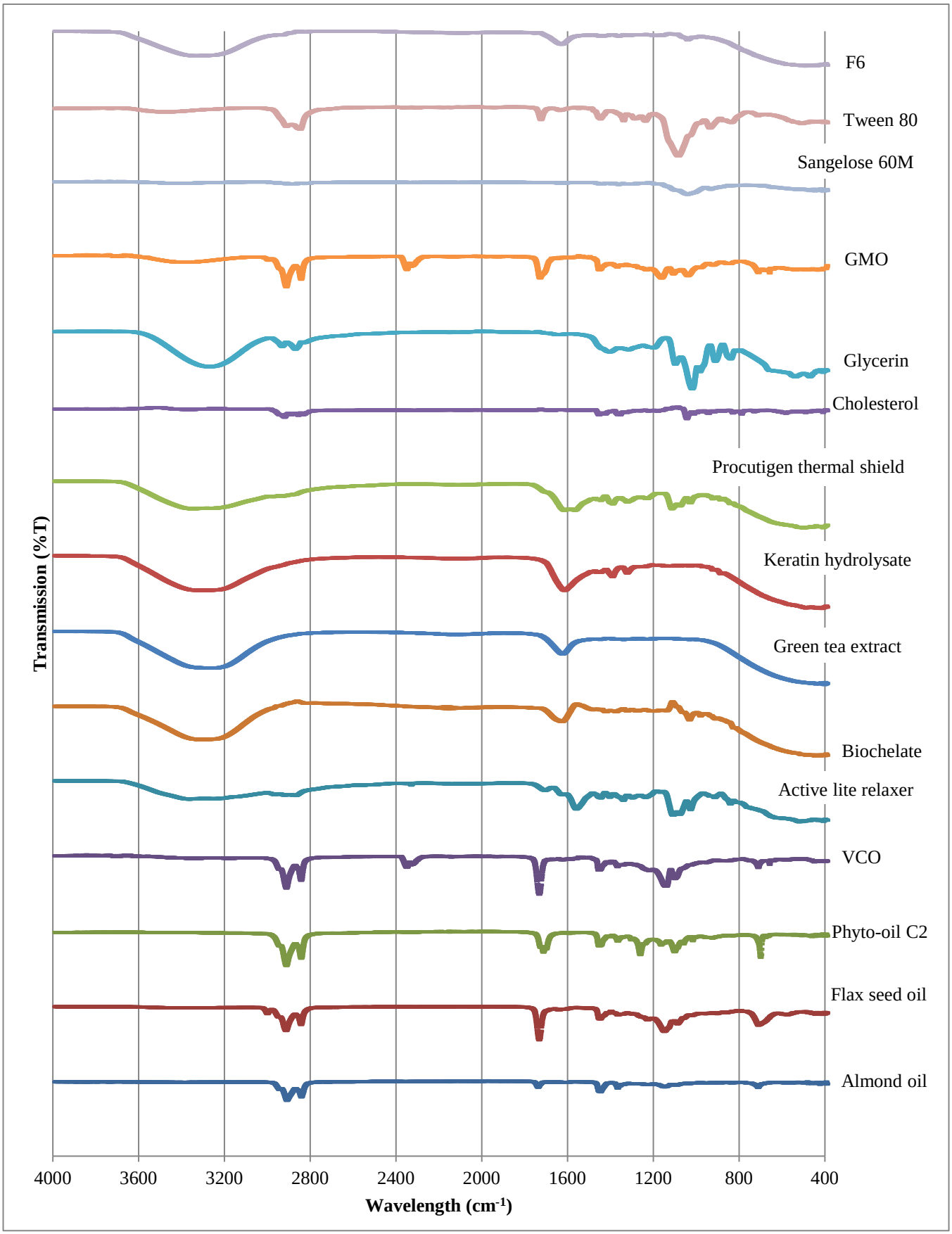

Figure 3: FTIR of F6 and its ingredients

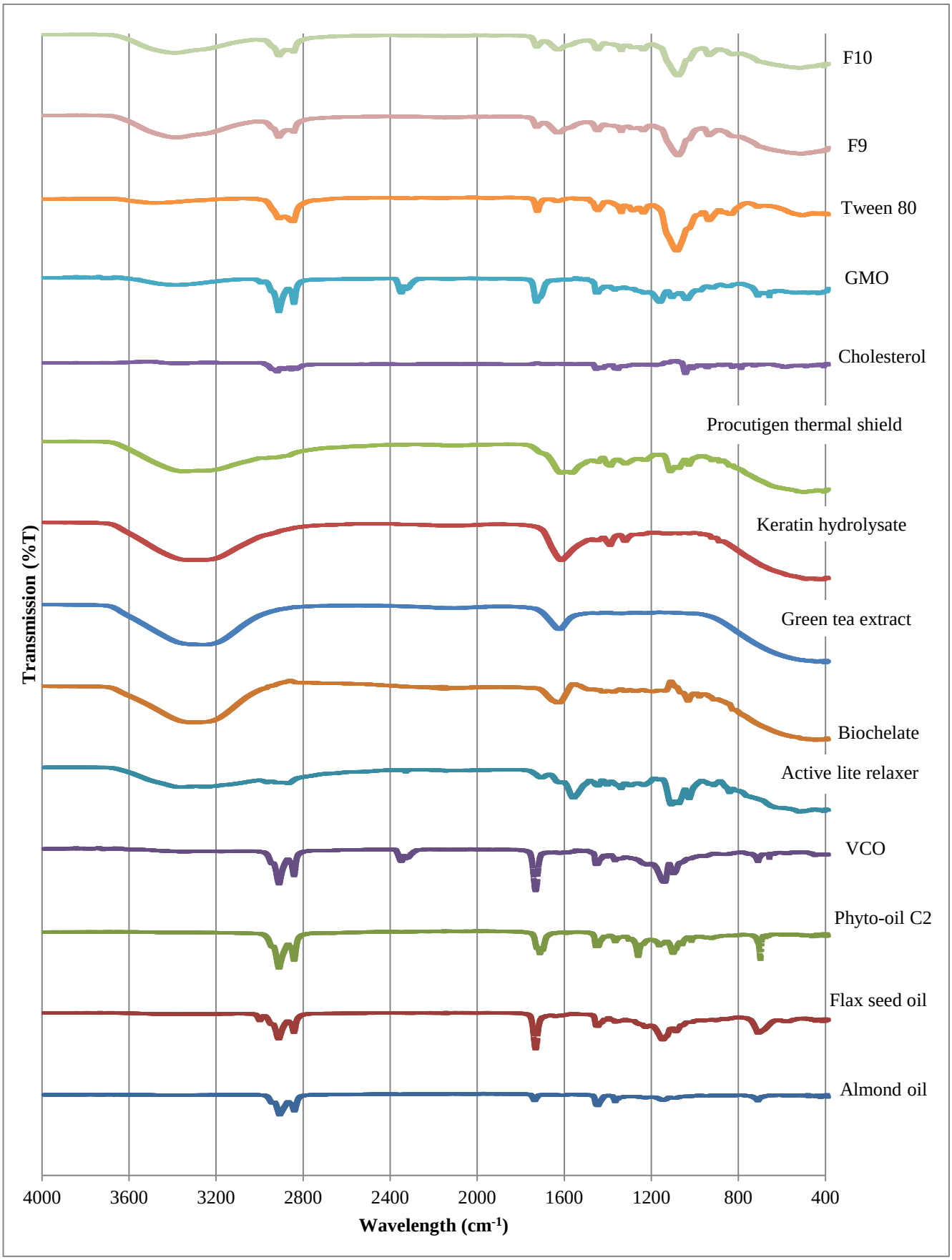

Figure 4: FTIR of F9, F10 and its ingredients

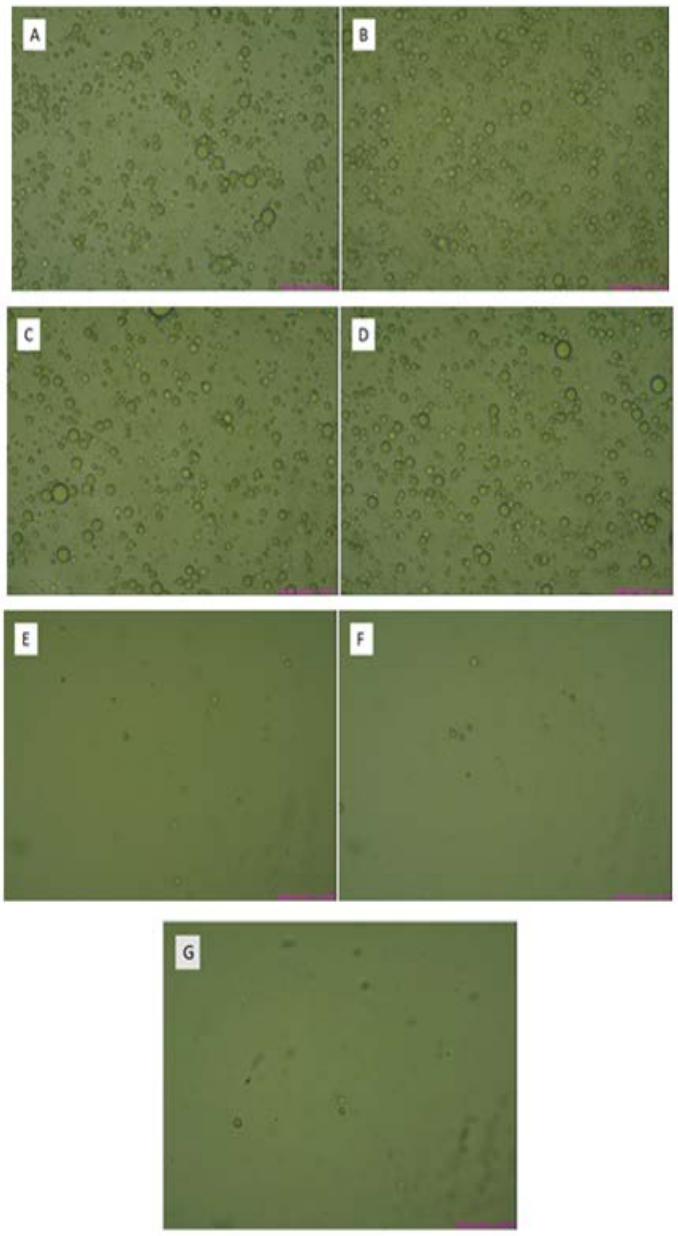

Figure 5. Microscopical image of formulations under 40× magnification(A–F1,B–F2, C–F3, D–F4, E–F6, F–F9, G-F10)

The detection of birefringent domains under polarized light microscopy is a well-established criterion for identifying liquid crystalline structures, particularly in surfactant-based systems stabilized with amphiphilic agents such as Glyceryl Monoleate (GMO) and Olivem® 2020 [12].

These phases offer several advantages, including thermodynamic stability, prolonged release of actives, and enhanced mechanical integrity of the gel matrix. Formulations F3, F4, and F10, which demonstrated clear birefringent textures, are likely to possess a lamellar or bicontinuous cubic phase structure. These phases are known for their ability to entrap both hydrophilic and lipophilic bioactives, offering controlled release and high biocompatibility.

Their optical anisotropy and fluid crystalline arrangement also enhance product aesthetics, such as gloss and texture, which are valued in consumer hair products [13]. Moreover, the observed birefringence correlates well with previous DLS findings: formulations with narrow PDI (<0.3) such as F6, F4, and F3 also showed strong liquid crystal characteristics, reinforcing the structural coherence and uniformity of these systems.

This alignment between optical and physicochemical data supports the presence of organized internal phases, contributing to long-term formulation stability. Conversely, the lack of birefringence in F2 and C1 may reflect incomplete emulsification, disordered mesophase arrangement, or surfactant deficiencies. Such disorganized matrices are prone to phase separation and rapid degradation of active ingredients [36].

In this study, polarized microscopy successfully confirmed the mesophase organization in key formulations. The presence of birefringent textures supports their potential as stable, visually appealing, and functionally superior hair gels, particularly suited for the controlled topical delivery of polyherbal bioactives.

Hair Thickness Enhancement

Hair thickness and mass were assessed using both diameter and weight measurements of non-bleached and bleached hair tresses across various formulations (F4, F6, F9) compared with a commercial standard and blank control.

As shown in Table 5, all tested formulations improved hair thickness and weight over time, with notable performance in F6 and F9. In non-bleached hair, F6 and F9 maintained consistent improvements, with final diameters reaching 0.05–0.06 mm and weight increases of up to 18.95 g after 24 hours. Similar trends were observed in bleached hair, where F4 and F6 significantly enhanced the diameter from 0.03 mm to 0.06 mm and hair weight from ~8.6 g to 10.06 g (F4) and 9.62 g (F6) at 24 h.

These values markedly outperformed the standard & blank, particularly in restoring structural integrity to chemically damaged hair fibers.

Table 5. Hair diameter and hair weight

Time	t=0		t=2		t=4		t=6		t=24	
	Hair weight (g)	Mean hair diameter (mm)	Hair weight (g)	Mean hair diameter (mm)	Hair weight (g)	Mean hair diameter (mm)	Hair weight (g)	Mean hair diameter (mm)	Hair weight (g)	Mean hair diameter (mm)
Non-bleached hair										
Blank	14.54	0.04 ± 0.02	14.39	0.05 ± 0.01	14.38	0.04±0.01	14.37	0.04 ± .01	14.41	0.05 ± 0.02
Std	21.32	0.06 ± 0.02	22.26	0.06 ± 0.02	21.91	0.06±0.01	21.78	0.06 ± .01	21.75	0.05 ± 0.01
F4	16.29	0.05 ± 0.02	17.73*	0.04±0.01*	17.61	0.05±0.01	17.57	0.05 ±0.01	17.58**	0.04 ± 0.01
F6	16.85	0.05 ± 0.01	17.53	0.05 ± 0.01	17.16	0.05 ±0.01	17.02	0.05 ± .01	16.95	0.06±0.02*
F9	19.58	0.05 ± 0.01	21.02*	0.06 ± 0.01	20.91	0.05 ±0.01	20.86*	0.05±0.01*	20.81	0.05 ±0.01
Bleached hair										
Blank	8.09	0.04 ± 0.01	8.06	0.03 ± 0.01	8.04	0.03 ±0.01	8.04	0.03 ± 0.01	8.05	0.03 ± 0.01
Std	8.26	0.04 ± 0.01	9.13	0.03 ± 0.01	8.89	0.04± 0.01	8.78	0.04 ± 0.01	8.69	0.04 ± 0.01
F4	8.77	0.03 ± 0.00	9.36	0.04 ± 0.01	9.26	0.05± 0.01	9.23	0.06±0.02*	9.18	0.06± 0.01*
F6	8.68	0.03 ± 0.01	9.21	0.04 ± 0.01	8.97	0.04± 0.01	8.87	0.04 ± 0.01	8.82	0.06± 0.01
F9	8.00	0.03 ± 0.01	8.98	0.05 ± 0.02	8.90	0.05± 0.01	8.87	0.06±0.01*	8.85	0.05± 0.02

All values represent mean ± SD (n = 3). *p < 0.05, **p < 0.01 vs. control (untreated hair).

Hair fiber diameter is a key determinant of cosmetic hair fullness and mechanical strength. The increase in hair thickness and weight observed here reflects not only temporary surface coating but also potential follicular hydration, cuticular reinforcement, and bioadhesive film formation, a phenomenon reported in phytogenic systems rich in lipids and polyphenols [40].

Formulation F6, which incorporated a liquid crystal base and uniform nano-dispersion (mean size ~186 nm, PDI 0.351), provided the highest uniformity in both bleached and non-bleached conditions. These results align with previous findings by Aliudin et al. (2024), who reported that lipid-rich gel systems may promote earlier and sustained anagen phase transitions in hair follicles through the formation of an occlusive film and the controlled delivery of botanical actives [41]. The hair thickness of formulations F4 and F6 showed a time-dependent trend, a notable increase at 2 hours, followed by a modest decrease at 24 hours. This pattern may result from the initial hydration and surface coating of the hair shaft by water-retentive bioactives, followed by partial dehydration and equilibrium of ingredient absorption over time. The final values at 24 hours remained higher than the baseline, suggesting persistent conditioning effects despite water loss [42].

Hair weight data further confirms the role of these formulations in moisture retention and tensile reinforcement, particularly in chemically stressed hair models. The improvement in bleached hair tresses was more pronounced for F4 and F6, implying a superior cuticle-binding and humectant effect, likely attributable to ingredients such as sweet almond oil, flaxseed oil, and protein hydrolysates. Similar findings were observed in gels based on Eclipta alba and Lippia nodiflora, which significantly increased follicular density and fiber weight in murine models [43]. Interestingly, F9, despite a higher PDI, performed comparably in non-bleached hair, suggesting that other factors, such as the presence of antioxidant-rich marula and Saccharomyces extract, may enhance follicular anchoring and shaft resilience.

In contrast, the commercial product showed only marginal improvements, suggesting that carrier design and ingredient synergy in the current study are more effective. Collectively, these findings suggest that polyherbal liquid crystal gels, particularly F6 and F4, significantly enhance hair fiber thickness and weight in both intact and damaged models. These effects appear to be mediated by a balance of hydrophilic and hydrophobic properties, microstructural stability, and sustained topical residence, making them promising alternatives to conventional chemical volumizers.

Anti-Frizz and Anti-Static Performance

The anti-frizz performance of polyherbal formulations F4, F6, and F9 was visually analyzed using both non-bleached and

bleached hair tresses, as shown in Figure 6. At baseline (t = 0), all hair samples appeared frizzy and uneven. After 6 hours of treatment under controlled humidity and temperature conditions, distinct improvements in hair alignment and smoothness were observed in the treated groups compared to the blank (A) and standard product (B).

Formulation F9 exhibited the most notable anti-frizz effect in non-bleached hair, resulting in enhanced manageability and visible reduction of flyaways, followed by F6 and F4. In bleached hair, F4 and F9 demonstrated superior performance, reducing frizz and improving hair cohesion, likely due to their rich lipid and film-forming properties.

The standard product (B) demonstrated limited reduction in frizz, especially on bleached hair. For the anti-static test depicted in Figure 7, rapid combing after drying induced visible static in the untreated (A) and standard (B) groups. In contrast, F4 exhibited the best anti-static effect on both non-bleached and bleached hair, maintaining hair alignment and reducing electrostatic repulsion.

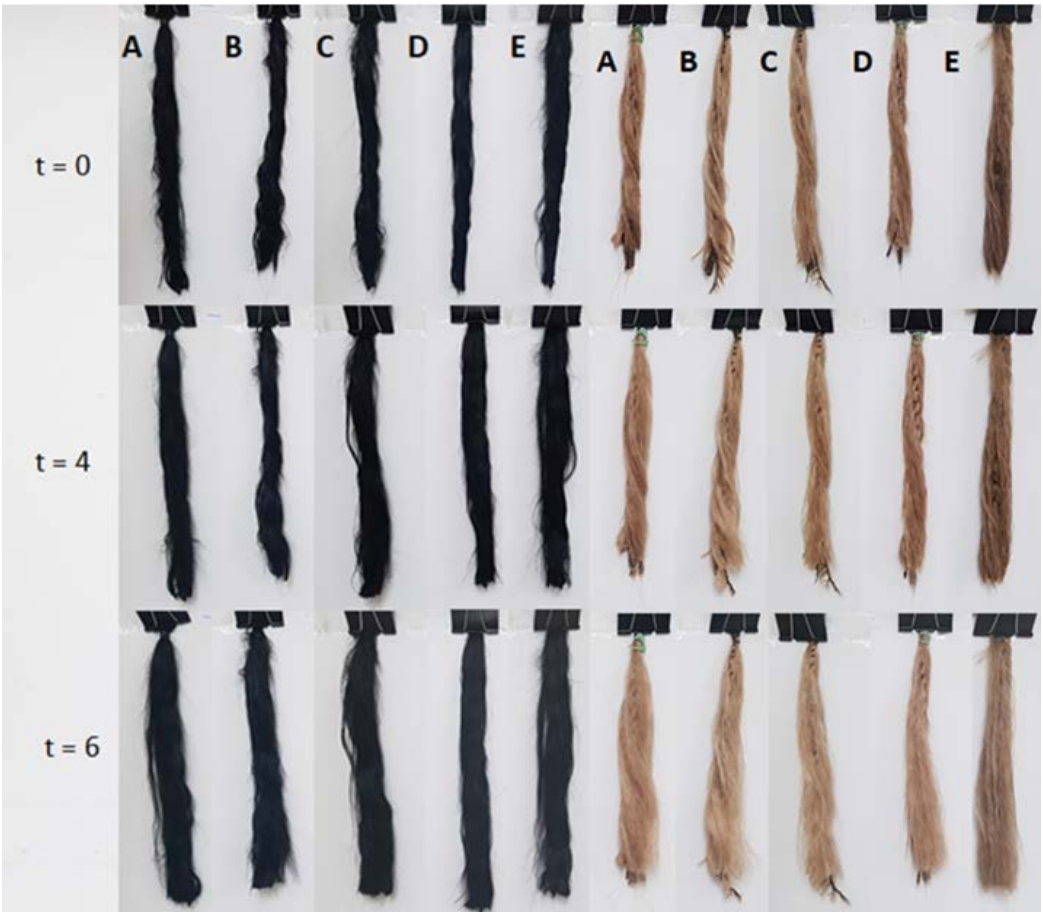

Figure 6. Anti-frizz analysis of non-bleached hair and bleached hair (A-Blank, B-Standard, C-F4, D-F6, E-F9)

F6 and F9 also showed visible improvement in static suppression compared to controls, with F6 performing better in non-bleached hair and F9 in bleached hair. These findings demonstrate the ability of formulations to form a protective, hydrating film over the hair shaft, improving both frizz and static control. Frizz and static are key indicators of hair fiber health and are closely associated with the surface properties and moisture balance of hair shafts.

Frizz is primarily caused by uneven moisture distribution and the lifting of hair cuticles, while static results from the loss of moisture and friction, especially after mechanical manipulation, such as combing [44]. Formulations F4 and F9 achieved enhanced anti-frizz performance due to their composition rich in polyunsaturated oils (almond oil, flaxseed oil, and marula), and proteinaceous actives such as keratin hydrolysate. These components coat the hair shaft, fill surface irregularities, and restore moisture barrier function, thereby minimizing cuticular lifting. In particular, keratin hydrolysates penetrate the cortex and enhance elasticity, which contributes to long-lasting hair alignment [45].

Figure 7. Anti-static analysis of non-bleached hair and bleached hair (A-Blank, B-Standard, C-F4, D-F6, E-F9)

The superior anti-static action of F4 may be attributed to the synergistic effect of film-forming agents (Tween-80 and GMO) and electrolyte-balancing minerals present in the Bio-Chelate complex. These compounds reduce surface charge accumulation by enhancing the conductivity of the hair surface, thereby preventing the buildup of triboelectric charge responsible for flyaway strands [40]. Interestingly, F6, although less lipid-rich than F9, demonstrated strong anti-static performance on non-bleached hair. This may be due to the structural contribution of sangelose 60M (methylcellulose derivative), which forms a consistent gel matrix over hair fibers, enhancing moisture retention and controlling static via occlusion [46]. In bleached hair, where cuticles are more porous and prone to disruption, the lipid-rich profile of F9 allowed better surface repair and antistatic protection. The relatively poor performance of the standard product (B) compared to experimental formulations highlights the role of botanical emollients and functional excipients in targeting hair shaft integrity. Recent literature suggests that hydrophobic actives and antioxidant-rich ingredients can reduce oxidative damage and improve surface smoothness, thereby enhancing frizz control [47]. Among the tested polyherbal hair gel formulations, F9 exhibited the most effective anti-frizz performance across both non-bleached and bleached hair types. This can be attributed to its rich composition of botanical oils such as almond, coconut, and flaxseed, which form an occlusive lipid film on the hair surface. These oils reduce hydrophilic swelling and seal cuticular gaps, thereby improving hair alignment and reducing flyaways [48]. F4 was superior in controlling static electricity in both hair types. This was likely due to its optimized blend of film-forming agents (like Tween 80 and Glyceryl Monooleate) and Bio-Chelate 5-PF, a complex of essential minerals known to improve hair conductivity and reduce electrostatic accumulation [48]. The consistent film deposition by F4 contributed to reduced triboelectric interactions during hair combing. Meanwhile, F6, although less rich in oils, performed well, particularly on non-bleached hair, due to its incorporation of Sangelose 60M (a methylcellulose derivative). This polymeric gel base is known to enhance moisture retention and create a uniform film over the hair cuticle, which minimizes static build-up and improves fiber cohesion under dry conditions. This aligns with findings on hydrogel-based delivery matrices for personal care use [49]. In contrast, the standard product showed limited efficacy in both anti-frizz and anti-static parameters, reinforcing the advantage of a polyherbal-lipid synergy in addressing surface irregularities and moisture dynamics of damaged or treated hair. These outcomes highlight the importance of tailored polyherbal and structural components in addressing hair fiber disorders through film-forming, hydrating, and electrostatic balancing mechanisms.

Antioxidant Activity

Figure 8 displays the DPPH radical scavenging activity for both the standard (Trolox) and the selected hair gel formulations (F4,

F6, and F9). Trolox exhibited a concentration-dependent increase in inhibition, achieving over 75% inhibition at just 20 µg/mL, with an estimated IC_{50} value of less than 10 µg/mL, confirming its potent antioxidant capacity. In contrast, polyherbal hair gel formulations (F4, F6, F9) exhibited low and relatively flat inhibition curves across the tested concentration range (100–500 µg/mL), yielding inhibition rates of less than 20% with no discernible dose-response pattern. These findings suggest that while the polyherbal gels do possess intrinsic antioxidant activity, they are markedly less potent than Trolox, a water-soluble vitamin E analog and standard antioxidant. The modest DPPH scavenging capacity observed in F4, F6, and F9 may stem from the presence of phenolic compounds and polyunsaturated fatty acids in flaxseed, coconut, and almond oils, which are known contributors to oxidative defense in topical applications [50]. However, the encapsulation of these lipophilic actives within a gel matrix may limit their immediate radical scavenging potential in solution-phase assays, such as the DPPH assay, a limitation previously noted in hydrogel-entrapped antioxidants [51]. Nevertheless, even moderate antioxidant protection is critical in hair care. Reactive oxygen species (ROS), often generated by UV exposure or pollution, compromise the integrity of the hair cuticle and accelerate protein degradation [52]. The incorporation of mild antioxidants may thus offer long-term protective benefits, especially when applied repeatedly. Moreover, lipid-rich matrices, such as those in F9, may act as physical barriers to oxidative stress, indirectly complementing chemical scavenging by limiting the penetration of ROS [30].

In summary, although the gels showed low in vitro radical scavenging compared to Trolox, their phytochemical composition and lipid barriers may still confer meaningful antioxidant protection during real-world application, especially in oxidative-stress-prone environments such as sun-exposed or chemically treated hair. While the current study demonstrated promising in vitro effects on hair texture and stability, it did not include cytotoxicity or skin irritation testing. Future work will focus on evaluating the dermal safety profile of the polyherbal gel through relevant cellular and skin models to support its potential for safe human application. Color evaluation was performed via visual observation. Instrumental colorimetric analysis using the CIELAB system was not included in this study but is planned for future formulation assessments to provide objective and reproducible data.

Stability test

Stability testing was performed under three storage conditions: ambient (25 ± 2 °C), refrigeration (4 ± 2 °C), and accelerated (40 ± 2 °C and 75 ± 5% relative humidity) using a Memmert stability chamber (Deutschland, Germany). Formulations were monitored at Days 0, 14, and 28 for changes in color, odor, pH, and spreadability. These parameters serve as key indicators of formulation consistency and shelf life under environmental stress conditions [53,54]. The stability of cosmetic formulations is crucial for ensuring their physicochemical integrity, user safety, and shelf life. In this study, the color, odor, pH, and spreadability of all polyherbal hair gel formulations were monitored under three different storage conditions: ambient temperature (25±2°C, no light), refrigeration (4±2°C), and accelerated aging (40±2°C and 75±5% RH) over 28 days (Tables 8–10). Most formulations, including F1, F2, F3, and F4, maintained their original color and odor under room and refrigerated conditions. However, under elevated temperature and humidity (40°C/75% RH), notable changes were observed. Formulations F6, F9, and F10 showed darkening of color and intensification of odor by Day 28. In contrast, F4 remained stable in both attributes across all three conditions, indicating better thermal and oxidative resistance of its constituents (Table 8). Initial pH values across formulations were within the physiologically acceptable range of 4.25–4.50. Upon storage, all formulations showed a slight decline in pH, especially under accelerated conditions. F6 demonstrated the best pH retention, remaining within 4.35 ± 0.04 (Day 28, 40°C), while F9 and F10 exhibited significant reductions below 4.3, possibly due to hydrolysis or degradation of labile herbal components (Table 9). This finding aligns with previous studies that report polyherbal gels with good buffering capacity exhibit stable pH profiles under stress conditions [41]. Spreadability ranged from 1.5–3.9 g·cm/sec across formulations. F6 exhibited consistent values (~2.1–2.2 g· cm-1 · sec-1) across all storage conditions, indicating robust viscoelastic behavior and an optimal emulsifier balance. In contrast, F10 and F9 showed larger variations, especially at 40°C, likely due to oil migration or phase instability (Table 10). Higher spreadability is desirable for easy application and uniform distribution on the scalp [55]. Both formulation constituents and environmental stress influence the physical and chemical stability of cosmeceutical gels. Formulations F4 and F6 emerged as the most stable across all parameters. F4's resilience can be attributed to the presence of antioxidant-rich ingredients such as green tea extract and keratin hydrolysate,

which inhibit oxidation-induced color or pH changes. F6's exceptional pH and spreadability stability suggest strong network structuring, likely aided by sangelose 60M and glycerin, which reduce water loss and maintain rheological consistency [56]. Accelerated degradation under high temperature and humidity, particularly in F10, aligns with general trends in herbal gel matrices, where volatile oils or phenolic degradation leads to odor intensification and visible discoloration. Studies by Ganji et al. (2022) similarly report that formulations with high essential oil content show a rapid decline in physical integrity under elevated storage conditions [57].

Table 8. Stability studies - Color and odor of formulations in different conditions

	Conditions													
			25±2°C and no light exposure				**4±2°C**				**40±2°C and 75±5% RH**			
	Day 0		**Day 14**		**Day 28**		**Day 14**		**Day 28**		**Day 14**		**Day 28**	
	Color	**Odor**	**Color**	**Odor**	**Color**	**Odor**	**Color**	**Odor**	**Color**	**Odor**	**Color**	**Odor**	**Color**	**Odor**
C1	White	++	White	++	White	++	White	++	White	+	White	++	White	+++
C2	White	++	White	++	White	++	White	++	White	+	White	+++	White	++++
F1	Light yellow	++	Light yellow	++	Light yellow	+++	Light yellow	++	Light yellow	++	Dark yellow	+++	Dark yellow	++++
F2	Light yellow	++	Light yellow	++	Light yellow	++	Light yellow	++	Light yellow	+	Dark yellow	++	Dark yellow	++++
F3	Yellow	++	Yellow	++	Yellow	+++	Yellow	++	Light yellow	+++	Dark yellow	++	Dark yellow	++++
F4	Light yellow	++	Light yellow	++	Light yellow	++	Light yellow	++	Light yellow	+	Dark yellow	++	Dark yellow	+++
F6	White	+	White	+	White	+	White	+	White	+++	White	+	White	+++
F9	Orange	+++	Orange	+++	Orange	+++	Orange	+++	Light orange	+++	Dark orange	+++	Dark brown	++++
F10	Orange-brown	+++	Orange-brown	+++	Orange	+++	Orange-brown	+++	Light orange	+++	Dark brown	+++	Dark brown	++

Table 9: Stability Studies pH of Formulation

	Mean pH in different conditions						
		25±2°C and no light exposure		**4±2°C**		**40±2°C and 75±5% RH**	
	Day 0	**Day 14**	**Day 28**	**Day 14**	**Day 28**	**Day 14**	**Day 28**
C1	7.34 ± 0.51	7.15 ± 0.07	7.17 ± 0.07	7.08 ± 0.06	7.13 ± 0.03	7.23 ± 0.01	7.10 ± 0.02
C2	6.74 ± 0.04	7.17 ± 0.03**	7.12 ± 0.02*	7.01 ± 0.03	6.88 ± 0.02	7.18 ± 0.03*	6.78 ± 0.06
F1	4.04 ± 0.02	4.46 ± 0.01**	4.49 ± 0.02**	4.10 ± 0.03	4.37 ± 0.01	4.42 ± 0.07*	4.29 ± 0.01
F2	4.02 ± 0.03	4.48 ± 0.02**	4.43 ± 0.02**	4.08 ± 0.02	4.37 ± 0.01	4.43 ± 0.05*	4.41 ± 0.03*
F3	4.12 ± 0.03	4.51 ± 0.01*	4.51 ± 0.06*	4.19 ± 0.05	4.43 ± 0.04	4.52 ± 0.00*	4.44 ± 0.02
F4	4.12 ± 0.02	4.50 ± 0.02*	4.52 ± 0.02**	4.22 ± 0.05	4.44 ± 0.01	4.43 ± 0.05	4.42 ± 0.00
F6	4.30 ± 0.27	4.91 ±0.07***	5.97 ±0.56***	5.61 ±0.38***	5.35 ±0.68***	5.41 ±0.51***	5.59 ±0.46***
F9	4.02 ± 0.10	4.51 ± 0.19**	4.43 ± 0.09**	4.04 ± 0.12	4.34 ± 0.02	4.40 ± 0.15*	4.52 ± 0.04**
F10	4.11 ± 0.09	4.36 ± 0.08	4.43 ± 0.12*	4.11 ± 0.07	4.35 ± 0.01	4.40 ± 0.19	4.63 ± 0.12**

Note: * for p < 0.05, ** for p < 0.01 and * for p < 0.001**

Table 10: Stability Studies - Spreadability of Formulations

	Mean spreadability (gcm/sec) in different conditions						
		25±2°C and no light exposure		4±2°C		40±2°C AND 75±5% RH	
	DAY 0	DAY 14	DAY 28	DAY 14	DAY 28	DAY 14	DAY 28
C1	0.755 ± 0.13	1.133 ± 0.17	1.195 ± 0.34	0.722 ± 0.10	0.884 ± 0.19	1.556 ± 0.28**	1.514 ± 0.35*
C2	2.395 ± 0.43	2.844 ± 0.24	3.534±0.37***	3.814±0.07***	3.977±0.83***	3.873±0.37***	3.918±0.04***
F1	3.511 ± 0.88	2.876 ± 0.38*	3.420 ± 0.67	2.753 ± 0.62*	3.348 ± 0.55	2.845 ± 0.08*	3.435 ± 0.77
F2	3.475 ± 0.31	2.640±0.22**	2.869 ± 0.30	2.867 ± 0.06	3.162 ± 0.30	2.990 ± 0.26	3.126 ± 0.27
F3	2.994 ± 0.24	2.809 ± 0.17	2.650 ± 0.16	2.922 ± 0.31	2.807 ± 0.01	2.686 ± 0.22	2.686 ± 0.09
F4	3.747 ± 0.33	3.183 ± 0.25	3.614 ± 0.19	3.229 ± 0.44	3.322 ± 0.52	3.895 ± 0.06	3.599 ± 0.29
F6	2.095 ± 0.20	1.812 ± 0.15	1.675 ± 0.06	2.105 ± 0.10	1.969 ± 0.05	1.693 ± 0.25	1.897 ± 0.02
F9	3.442 ± 0.16	2.867 ± 0.27	3.276 ± 0.38	2.942 ± 0.28	2.939 ± 0.83	3.351 ± 0.30	3.488 ± 0.36
F10	4.031 ± 0.36	3.327 ± 0.20*	3.802 ± 0.22	3.576 ± 0.24	4.076 ± 0.27	3.633 ± 0.31	3.861 ± 0.15

Note: * For p < 0.05, ** For p < 0.01 and * For p < 0.001**

CONCLUSION

This study successfully demonstrated the formulation, characterization, and in vitro evaluation of novel liquid crystal-based polyherbal hair gels integrating herbal actives with advanced delivery systems. Among the ten formulations, F4 and F6 consistently outperformed others in terms of physicochemical stability, hair enhancement properties, anti-frizz and anti-static performance, and overall formulation resilience. Notably, F6 exhibited optimal nanoscale characteristics with superior uniformity (PDI < 0.4) and robust zeta potential, ensuring colloidal stability and efficient delivery. FTIR and microscopic analyses confirmed the structural integrity and liquid crystalline mesophase formation, which are essential for sustained topical performance.

Furthermore, hair tress studies highlighted significant improvements in fiber thickness, manageability, and surface aesthetics, especially in damaged (bleached) models, validating the therapeutic and cosmetic potential of these formulations. The polyherbal gel exhibited modest antioxidant activity, as indicated by the DPPH assay results. While the observed scavenging effect suggests potential free radical neutralization, further assays (e.g., FRAP or lipid peroxidation) are warranted to confirm broader antioxidant efficacy. Stability data confirmed the robustness of F4 and F6 under varied environmental conditions, underscoring their commercial readiness.

In summary, this research supports the use of liquid crystal systems as a promising platform for delivering polyherbal actives in hair care. The integration of lipidic nanostructures with plant-derived bioactives represents a scientifically and consumer-appealing approach to managing hair damage, enhancing cosmetic quality, and addressing scalp health holistically. However, in vivo and long-term clinical studies are necessary to confirm efficacy and safety in human populations. Future studies will focus on conducting in vivo efficacy assessments, dermal safety evaluations, and mechanistic investigations, including hair follicle penetration studies, to support the translational potential of the polyherbal LC-based hair gel.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Sheba R. David conceptualized the study and supervised the research. Umi Haida, Nadia Haji, and Mohamed Jefri conducted the experimental work and performed the data analysis. Rajan Rajabalaya contributed to the design of the methodology, drafted the manuscript, and oversaw the overall project administration. All authors reviewed and approved the final manuscript.

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