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Formulation Optimisation of *Erythrina variegata* potential Wound-Healing Cream

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Abstract

The increasing reliance on synthetic compounds in wound-healing and cosmetic formulations has raised concerns regarding adverse effects and limited therapeutic functionality, underscoring the need for optimised plant-based alternatives with intrinsic bioactivity. *Erythrina variegata*, a medicinal plant rich in flavonoids and phenolic compounds, exhibits antioxidant and antimicrobial properties but remains underexplored in systematically optimised topical formulations. This study aimed to design and optimise a cosmetic cream incorporating bioactive extracts from *E. variegata* for wound-healing application, utilising a two-step Design of Experiment (DOE) approach via *Design-Expert®*. In the first phase (DOE-1), the base cream was optimised for maximum stability, resulting in an optimal formulation consisting of 10 wt% virgin olive oil, 90 wt% deionised water, and 2.75 phc Aquagel35. The second phase (DOE-2) optimised extract incorporation, identifying 0.5 phc of *E. variegata* extract and mixing condition of 5000 rpm for 2 minutes as optimal. Comprehensive physicochemical analyses, including accelerated stability test, viscosity determination, and droplet size measurements, confirmed that the optimised cream maintained sufficient consistency and stability. Numerical optimisation demonstrated that the formulations selected exhibited the highest structural integrity among the experimental sets. Rather than generating a new formulation based solely on the theoretical optimum, experimental runs from the DOE matrix most closely aligned with the optimised parameters were selected for evaluation. These findings demonstrate that the systematic DOE approach effectively produces a stable, high-quality topical formulation suitable for further medicinal application.

1. Introduction

Cosmetic creams are a crucial element in skincare in which it provides protection against skin's external and internal adverse while simultaneously delivering therapeutic benefits to help enhance skin's general condition [1]. Recent development has considerably broadened the range of skincare products due to the rise of cosmeceuticals. This term usually referred to a product that utilised the incorporation of bioactive compounds that supports both medicinal and aesthetic benefits for skins. A distinct contrast between conventional cosmetics and cosmeceuticals can be distinguished by the main objective of the products; whether it is concerned with appearance and external wellbeing of skins only or if it purposely targets specific issues like inflammation and hyperpigmentation by penetrating deeper layers of the skin [2].

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The search for effective natural alternatives to synthetic compounds is driven by concerns over adverse effects such as toxicity, allergic responses, and antibiotic resistance often found in commercial wound-healing formulations [3]. Among potential botanical sources *Erythrina variegata*, locally known as "Dedap", has emerged as a promising candidate. Its bioactive molecules, especially flavonoids, alkaloids, and phenolic compounds offer significant potential benefits for anti-aging, wound healing, and protection from microbial infections [4]. While extensively utilised in traditional medicine, *E. variegata* had not been widely integrated into standardised cosmetic formulations.

Furthermore, conventional wound care techniques like bandages and patches focused solely on moisture retention and physical protection which mean they do not possess the antioxidant or antibacterial properties that are able to speed up the wound-healing process and stave off infections [5,6]. In a lot of cases, herbal ointments lack thorough formulation and optimisation to guarantee consistent therapeutic effects, even if some have demonstrated advantages for wound healing [7].

To address these needs, this study investigates the systematic development of a *E. variegata* wound-healing cream using two-step DOE methodology via Design-Expert. The goal is to determine the composition of cream's ingredients such as virgin olive oil, deionised water, and Aquagel35 to produce the most stable base cream on top of establishing the optimum loading of *E. variegata* extract into the base cream matrix without compromising its stability and physicochemical properties.

2. Methodology

This section outlines the methodology employed to design and develop the proposed optimised *E. variegata* extract cream using two-step DOE via *Design-Expert* software. DOE-1 focused on determining optimal loading of each cream ingredients; virgin olive oil, deionised water, and Aquagel35 (surfactant) to produce the most stable base cream. DOE-2 on the other hand centered on determining the ideal loading of *E. variegata* extract to be incorporated into base cream complete with the mixing speed and duration without disturbing the cream's stability and physicochemical properties. Both stability test and physicochemical analysis, including viscosity determination and droplet size measurement, were repeated for both DOE-1 and DOE-2 to ensure consistency across the optimisation phases. After the evaluation, the experimental data for the observed responses were keyed back into *Design-Expert* software. Utilising experiment framework (RSM), the software then generated optimised numerical solutions to identify the most favorable formulation compositions. The goal was to develop a stable base cream (DOE-1) and produce *E. variegata* extract cream with optimal extract loading while identifying the best mixing speed and duration of extract incorporation (DOE-2) while maintaining cream's stability and integrity.

2.1 Design of Experiment (DOE) Strategy

This research employed a systematic two-step Design of Experiment (DOE) approach to design and develop an optimized *E. variegata* extract cream using Design-Expert software. The initial phase, DOE-1, focused on determining the optimal loading of base cream ingredients; virgin olive oil (VOO), deionised water, and Aquagel35, to establish a stable base cream. The second phase, DOE-2, aimed to identify the ideal loading of *E. variegata* extract, as well as the mixing speed and duration required to integrate the active components without compromising the formulation's structural integrity or physicochemical properties. By inserting factors and ranges detailed in **Table 1** into the software, 19 (DOE-1) and 16 (DOE-2) experimental runs were generated as shown in **Figure 1**.

Table 1 *Factors and ranges used for both DOE-1 and DOE-2 to generate formulations for experimental runs*

Phase	*Factor*	*Range*	*Unit*
DOE-1	*Loading of VOO*	*5 – 15*	*wt%*
	Loading of deionised water	*85 – 95*	*wt%*
	Loading of Aquagel35	*0.25 – 3.00*	*phc*
DOE-2	*Loading E. variegata* extract	*1-10*	*phc*
	Speed of mixing	*1000 – 5000*	*rpm*
	Duration of mixing	*5 – 15*	*Minute*

Run	Factor 1 A:loading of VOO wt%	Factor 2 B:loading of DI water wt%	Factor 3 C:loading of surfactant phc
1	10	90	1.75
2	5	95	0.5
3	10	90	3
4	10	90	2
5	10	90	0.5
6	10	90	1.25
7	10	90	0.75
8	10	90	2.5
9	5	95	1.75
10	10	90	1.5
11	10	90	0.25
12	10	90	1
13	15	85	1.75
14	15	85	3
15	14	86	1.75
16	10	90	2.75
17	5	95	3
18	15	85	0.5
19	6	84	1.75

(a)

Run	Factor 1 A:Loading of Erythrina phc	Factor 2 B:Mixing speed rpm	Factor 3 C:Duration mixing min
1	3	5000	2
2	3	1000	2
3	2	2550	2
4	3	2550	1
5	1	2550	1
6	2	1000	3
7	1	2550	3
8	2	5000	1
9	2	2550	2
10	1	1000	2
11	3	2550	3
12	2	5000	3
13	2	1000	1
14	1	5000	2
15	0.5	1000	2
16	0.5	5000	2

(b)

Fig. 1 *DOE matrix generated by Design-Expert for (a) DOE-1; (b) DOE-2*

2.2 Cream Formulation

2.3 Response Evaluation

Following generation of the experimental matrix using Design-Expert® software, all formulations were subjected to accelerated stability testing. Formulations exhibiting instability, including phase separation or creaming, were excluded from the Design of Experiment (DOE) analysis. Only formulations that maintained a uniform emulsion were advanced to final response evaluation, including viscosity measurement and droplet size analysis.

2.3.1 Accelerated Stability Test

To optimize experimental efficiency and minimize the sample size for subsequent analyses, an accelerated stability test was conducted using the centrifugation method to pre-screen and eliminate unstable formulations. For each experimental run, 1 g of the cream sample was loaded into a microtube and subjected to centrifugation at a controlled temperature of 25°C. The samples were processed at a speed of 3000 rpm for a duration of 30 minutes utilising a microcentrifuge [8].

Physical stability was quantified by calculating the emulsion stability index percentage based on the degree of phase separation observed (Eq 1).

$$\text{Emulsion stability index (\%)} = \frac{\text{height of emulsion separation}}{\text{total height of emulsion}} \times 100\% \quad (1)$$

2.3.2 Viscosity Test

The rheological properties of the formulated creams were characterized to evaluate their consistency and flow behavior under stress. Viscosity data was extracted from measurements performed using a Discovery HR-1 hybrid rheometer. The instrument was equipped with a 60 mm stainless steel cone geometry featuring a 2° cone angle.

To ensure accuracy and simulate application on human skin, all tests were conducted at a constant temperature of 32°C ± 1°C with a gap size of 0.25 mm [9]. The analysis employed a stress range of 0.001 Pa to 20 Pa, performed with a ramp time of 2 minutes and a decade of 10 [9]. This standardized approach allowed for the determination of the cream's structural robustness and spreadability, ensuring the formulations maintained the necessary consistency for topical use.

2.3.3 Droplet Size Measurement (with slight modification to Boxall et al., 2009)

The internal microstructure of the emulsions was evaluated through microscopic analysis to determine droplet size distribution. A small quantity of the cream was placed onto a microscope slide and secured with a coverslip to ensure the formation of a uniform, thin layer. The samples prepared were then observed under an inverter microscope at a magnification suitable for clear droplet visualization [10].

To ensure that the findings were both consistent and representative, digital image processing was used in conjunction with optical microscopy. High-resolution images of the droplet distribution were captured via an inbuilt camera and exported in JPEG format. These images were subsequently analyzed using ImageJ software, where the droplet boundaries were manually thresholder to ensure precise measurement.

The "measure" function was employed to determine the diameter of multiple droplets per experimental run, and the average droplet size (expressed in μm) was recorded as the final response result. This rigorous quantification of droplet diameter served as a critical indicator of the emulsion's physical stability and the efficiency of the homogenization process.

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3. Results and Discussion

3.1 Cream Formulation

The formulation of the *E. variegata* extract cream was conducted in two distinct experimental phases, during which the physical characteristics of the resulting mixtures varied according to the formulation ratios and process parameters generated by Design-Expert® (**Figure 1a**). Both base and extract cream were developed by utilising a cold-method direct emulsification technique. In the first phase (DOE-1), the formulation consisted of a deionised water aqueous phase, a virgin olive oil (VOO) oily phase, and Aquagel35, which served as the polymeric surfactant. To ensure precision, each formulation was prepared by weighing all components to achieve a total sample mass of 50 grams, strictly adhering to the generated design matrix. 19 base cream sample was produced by employing high-speed homogenization at 5000 rpm for 10 minutes. At this phase, mixing speed and duration was specified to focus on the physicochemical properties of the cream resulted from different composition of cream's ingredient only. As depicted in **Figure 2a s**ome base cream sample immediately transformed into smooth, brilliant white semi-solid emulsion with a consistent texture, while others exhibited poor thickening or "watery" characteristics where surfactant failed to adequately bridge the phases. These variations in the fresh-produced base cream samples provided the baseline for elimination process through the subsequent stability screening.

As for the second phase (DOE-2), *E. variegata* extract was introduced into the optimised base cream after DOE-1 process was done. The incorporation of bioactive extract initially created a visible third phase which manifested as a dark botanical liquid resting on the white cream base. As the 16 extract samples were produced according to specified parameters as shown in **Figure 1b**, which varied extract loading, mixing speed, and mixing duration, a notable colour transition occurred. The formulations shifted from a pure white to a uniform, golden-brown hue. In experimental runs employing higher mixing speeds (up to 5000 rpm), the resulting creams appeared dense and highly uniform, whereas runs at lower mixing speeds or shorter mixing durations showed uneven colour distribution or localised "leaching" of the extract. These extract cream samples represented the complete integration of the bioactive components of *E. variegata* within the base cream matrix prior to the application of external stress via centrifugation for accelerated stability test. Same as DOE-1, standardised 1 gram of extract cream samples from DOE-2 was also put on display on a glass for texture comparison which are shown in **Figure 2b.**

(a)

(b)

Fig. 2 Texture or physical structure of each (a) base cream sample; (b) extract cream sample

3.2 Accelerated Stability Test

The physical stability of the formulated creams was first evaluated using accelerated centrifugation to predict long-term shelf-life and emulsion integrity. The centrifugation test was conducted to simulate the effect of prolonged gravitational forces within a short period [10]. 20 to 30 minutes of stirring at 3500 rpm could be said to be equivalent to gravity for ±1 year [12]. The degree of integrity for each formulation was quantified using the emulsion stability index (Eq 1), which measured the extent of separation following high-speed stress.

According to a study by Khan et al., "creaming" is a natural phenomenon in biphasic system and serves as a primary indicator of destabilisation [13]. For DOE-1, which focused on the base cream, several formulations exhibited immediate phase separation or "creaming". This instability was primarily observed in runs with a high water-to-oil ratio paired with insufficient Aquagel35 loading, where the surfactant concentration was inadequate to form a robust interfacial film around the oil droplets. In an effort to ensure the reliability of the optimisation process, these unstable base cream samples were eliminated. The instability observed in samples #2, #5, #8, #11, #13, #17, and #18 was consistently correlated with the lower threshold of surfactant loading (Aquagel35) or an imbalance in the oil-to-water ratio. These results confirm that a minimum surfactant concentration is required to overcome the interfacial tension and prevent the coalescence of VOO droplets under centrifugal stress.

In DOE-2, the integration of the *E. variegata* extract introduced new variables into the emulsion system. It was observed that the mixing speed and duration played a critical role in stabilization. Runs performed at lower mixing speeds such as 1000 rpm were more prone to leaching or colour sedimentation as seen in **Figure 3d** during the stability test compared to those processed at the optimized speed of 5000 rpm. The high-speed homogenisation provided sufficient mechanical energy to finely disperse the bioactive extract within the base cream. **Table 2** summarise the overall results for accelerated stability test using centrifugation method.

Ultimately, the accelerated stability test served as an effective primary filter, ensuring that the numerical solutions generated by the Response Surface Methodology (RSM) were derived from a pool of robust, physically viable candidates. This rigorous screening process confirmed that the final optimized formulations possess the structural integrity required for consistent topical delivery.

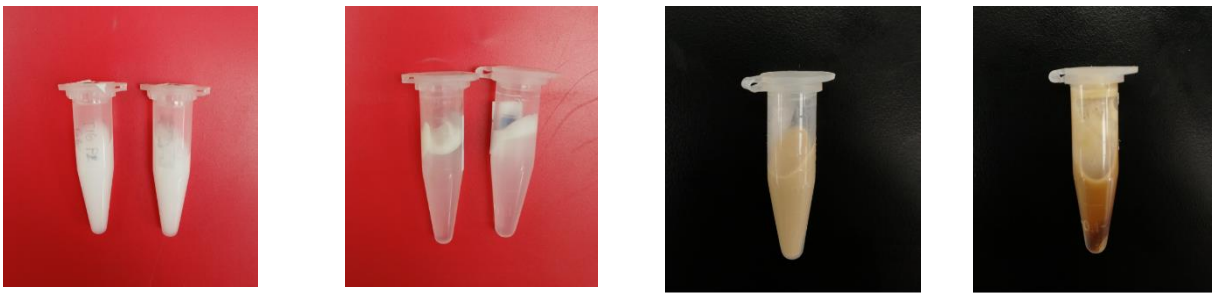

Fig. 3 *Physical observation comparison for (a) stable base cream; (b) unstable base cream; (c) stable extract cream; (d) unstable base cream*

Table 2 Summary of accelerated stability result

Phase	Sample no	Physical Observation	Stability Status
DOE-1	1, 3, 4, 9, 10, 12, 14, 15, 16, 19	Homogenous, no phase separation	Stable
	2, 5, 8, 11, 13, 17, 18	Phase separation observed (creaming)	Unstable (Eliminated)
DOE-2	5, 7, 10, 12, 14, 15, 16	Homogenous, no phase separation	Stable
	1, 2, 3, 4, 6, 8, 9, 11, 13	Phase separation observed (creaming)	Unstable (Eliminated)

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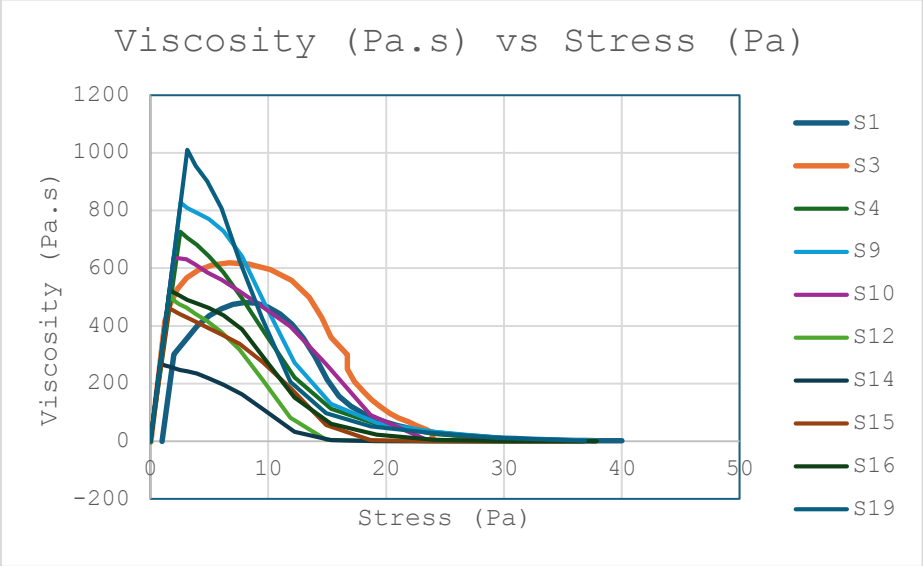
3.3 Viscosity Test

The rheological profiles of the formulations were evaluated to determine the flow behavior, structural integrity, and consistency of both base cream (DOE-1) and the functionalized extract cream (DOE-2). Viscosity is a critical parameter influencing spreadability, physical stability, and consumer acceptability [12]. Evaluations were conducted at 32°C to mimic skin application conditions, with viscosity data standardized at a stress of 4 pa for DOE-1 and value of initial yield stress was utilized for DOE-2 [15].

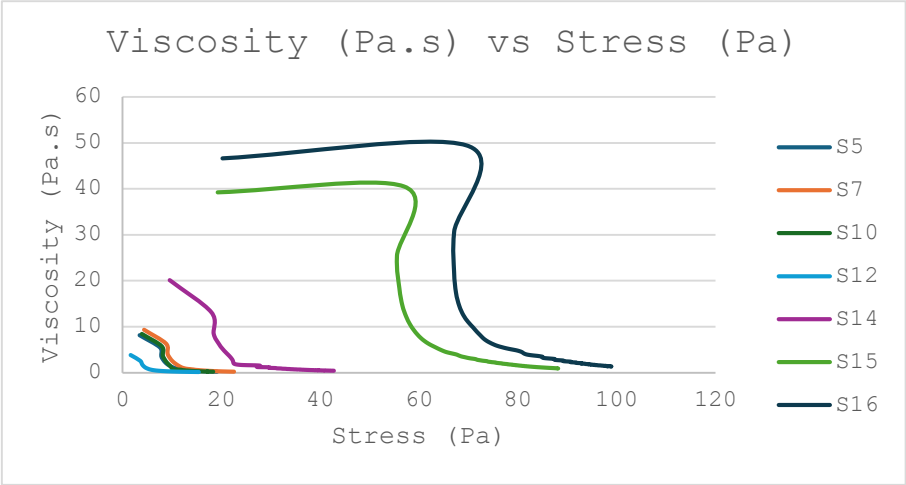
Standardised viscosity data for all base cream samples are presented in Figure 4. The flow curves in Figure 4a revealed that all samples exhibited non-Newtonian, shear-thinning (pseudoplastic) behavior. The formulations initially showed a viscosity peak followed by structural breakdown under increasing stress. Base cream sample #19 demonstrated the highest peak viscosity, indicating a robust internal network that requires a significant force to disrupt which potentially reduces its pourability. In contrast, sample #14 exhibited the lowest viscosity, suggesting a minimal flow resistance and higher spreadibility. Intermediate samples such as sample #12 and #15, represented an optimal balance between structural stability and ease of application [16]. A sharp viscosity decline observed between 10 Pa and 25 Pa signifies the yield point where shear force overcomes the semi-solid matrix [15]. This transition is attributed to the deformation of internal oil droplets from spherical to ellipsoidal shapes, which align with the flow direction and facilitate fluid layer sliding [18].

Following the stability screening of the *E. variegata* extract cream, seven viable formulations were advanced for rheological assessment. As depicted in **Figure 4b**, viscosity and yield stress were highly dependent on processing parameters, specifically mixing speed and duration. The initial "static" yield stress was utilized as the primary metric, representing the magnitude of internal forces maintaining the surfactant-gel network and oil droplets in a fixed arrangement [19].

A high initial yield stress (Figure 4b) indicates a robust internal skeleton capable of supporting dispersed droplets against gravitational pull [20]. Based on Figure 4.8, extract sample #16 maintained its viscosity under increasing stress significantly longer than other samples, confirming its structural bonds were the strongest in the set. This high yield stress serves as a benchmark for ensuring the cream remains shelf-stable while remaining pleasant for topical application. These findings suggest that the mechanical energy provided during homogenization (DOE-2) effectively integrated and dispersed the bioactive extract successfully without compromising the structural integrity established in DOE-1.



(a)



(b)

Fig. 4 Rheological profile: graph viscosity against yield stress for (a) base cream sample (DOE-1); (b) extract cream sample (DOE-2)

3.4 Size Droplet Measurement

The internal microstructure of the emulsions was evaluated through microscopic analysis to determine the droplet size distribution, which serves as a critical indicator of the cream's physical stability and the efficiency of the homogenization process [21]. Droplet diameter significantly influences the texture, appearance, and the rate of bioactive release in topical formulations.

Digital image processing using ImageJ software was employed to quantify the average droplet size (μm) of the stable base cream formulations. The results indicated that the mean droplet diameter varied according to the specific ratios of virgin olive oil (VOO) and surfactant loading. Formulations with an optimized concentration of Aquagel35 exhibited smaller, more uniform droplets, suggesting a more efficient reduction in interfacial tension during the cold-method emulsification. Samples with smaller droplet sizes generally correlated with the higher viscosity values. This is attributed to the increased surface area of the dispersed phase, which enhances droplet-droplet interactions and strengthens the resistance to flow [18]. Conversely, larger droplets observed in some runs indicated a higher risk of coalescence, which had been the primary cause for the elimination of unstable samples during the accelerated stability screening.

For the *E. variegata* extract cream, droplet size was used to evaluate how mixing speed and duration influenced the integration of the bioactive components. The results for the seven stable samples showed that the mechanical energy provided during homogenization was the dominant factor in achieving microstructural uniformity. Extract sample #16, which displayed the highest structural integrity in the rheological tests, also exhibited the most refined and consistent droplet distribution. The high-speed homogenization at 5000 rpm provided sufficient shear to break down the bioactive extract and oil phases into a fine, homogeneous dispersion. This refined microstructure explains the high initial yield stress of sample #16 which proven that smaller droplets provide a higher packing density within the surfactant-gel network, thereby supporting the internal skeleton against gravitational pull [20].

The digital image processing confirmed that the incorporation of *E. variegata* extract did not lead to significant droplet aggregation in the stable runs. Instead, the optimised mixing parameters ensured that the phytochemicals were effectively encapsulated within the emulsion matrix, resulting in a stable, golden-brown cream with a droplet size profile suitable for consistent topical delivery.

3.4.1 Numerical Solution

The final phase of the systematic design utilized the Response Surface Methodology (RSM) framework in *Design-Expert* to generate optimized numerical solutions for both DOE-1 and DOE-2. This goal-driven approach identified the specific ingredients' loading and processing parameters required to maximize formulation desirability while maintaining structural integrity.

For the base cream, the software generated several solutions with a maximum desirability score of 1.000. The selected formulation (Solution #1) comprised 14.25 wt% virgin olive oil (VOO), 92.37 wt% deionized water, and 0.669 phc of Aquagel 35. This composition was predicted to yield a standardized viscosity of 796.93 cP and a

highly refined droplet size of 0.031 μm. These parameters successfully balanced the oily and aqueous phases within a stable surfactant network, providing the optimal foundation for extract incorporation.

Following the establishment of the optimized base, DOE-2 was executed to refine the integration of the *E. variegata* extract. The numerical solutions indicated that a 1.000 phc extract loading was the optimal concentration for maintaining bioactive efficacy without destabilizing the emulsion. The selected processing parameters (Solution #1) included a mixing speed of 5000 rpm and a mixing duration of 1 minute, achieving a desirability score of 0.807. For DOE-1, Sample #16 (comprising 10 wt% VOO, 90 wt% deionized water, and 2.75 phc Aquagel 35) was selected as the most stable foundation. In DOE-2, sample #16 was again identified as the superior choice, utilizing an *E. variegata* extract concentration of 0.5 phc processed at a mixing speed of 5000 rpm for 2 minutes. These selections ensure that the final cream possesses a robust internal skeleton and refined droplet distribution, providing the optimal consistency for topical application. The convergence of these parameters confirms that the two-step DOE approach produces a stable, golden-brown topical cream suitable for wound-healing applications.

4. Conclusion

This study successfully optimised a wound-healing cream containing *Erythrina variegata* extract using a two-step Design of Experiment (DOE) approach. DOE-1 identified a stable base carrier composed of 10 wt% virgin olive oil, 90 wt% deionized water, and 2.75 phc of Aquagel35. In DOE-2, an optimal 0.5 phc extract concentration was successfully integrated using a homogenization speed of 5000 rpm for 2 minutes, ensuring a uniform, stable distribution.

Accelerated stability testing acted as a critical filter, eliminating seven unstable base cream samples and nine unstable extract samples to ensure only robust formulations remained. Rheological analysis confirmed that all stable samples displayed non-Newtonian and shear-thinning, which is essential for effective topical application. Notably, extract sample #16 demonstrated superior mechanical strength with the highest initial yield stress, indicating a robust internal skeleton capable of resisting gravitational stress. This structural integrity was further validated by microscopic analysis, which revealed a refined and consistent droplet distribution in sample #16.

These findings confirm that the systematic DOE methodology effectively produces a stable, high-quality topical formulation. The optimised *E. variegata* cream maintains the necessary microstructural integrity and consistency for standardized medicinal use in wound-healing applications.

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