

Formulation and In Vivo Study of A Liquid Crystal Containing Propolis Extract for Wound Healing Treatment

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Abstract

Impaired wound healing remains a major clinical challenge, necessitating the development of effective and biocompatible topical therapies. This study aimed to develop and evaluate a propolis extract based liquid crystalline (LCPE) cream for wound-healing applications. Propolis was extracted with 70% ethanol using ultrasound-assisted extraction (UAE), yielding 21.21% extract with a total phenolic content (TPC) of 249.6 ± 20.8 mg GAE/g and antioxidant activity (IC_{50}) of 504 ± 12 ppm. LC-MS/MS analysis identified the major bioactive compound as mangostin. Liquid crystalline cream bases were optimized using a one-factor-at-a-time (OFAT) approach with varying concentrations of cetostearyl alcohol. The formula demonstrated suitable pH (4-7), viscosity (10,402–40,742 cps), spreadability, adhesiveness, and physical stability. Polarized light microscopy (PLM) confirmed the formation of anisotropic liquid crystalline structures, while DSC analysis indicated successful incorporation and thermal compatibility of the extract within the system. In vivo wound-healing evaluation using a rabbit incisional wound model demonstrated that LCPE significantly accelerated wound closure and reduced inflammation compared to untreated control group. Among the tested concentrations (1%, 2.5%, and 5%), LCPE 2.5% exhibited the most effective healing performance, as evidenced by faster wound closure and lower REEDA scores. In conclusion, LCPE represents a stable, skin-compatible, and promising topical delivery system for wound healing.

Keywords

Liquid Crystalline System, Propolis Extract, Wound Healing, Lipid-Based Delivery Topical Formulation

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1. INTRODUCTION

Under normal circumstances, wound healing is a highly coordinated and dynamic process involving overlapping phases of inflammation, cell migration and proliferation, extracellular matrix (ECM) synthesis, and tissue remodeling (Li et al., 2024; Yang et al., 2023). As the skin serves as the body's primary barrier against environmental factors, the unbalanced mechanisms to restore skin integrity may result in burns, diabetic ulcers, psoriasis, pressure ulcers, and chronic wounds, which not only cause pain but also impair quality of life (Conceição et al., 2022; Karakas et al., 2023). Approximately 8.2 million individuals suffer from impaired wound healing due to physical, chemical, or disease-related factors, with or without infection, resulting in healthcare costs estimated at USD 20–90 billion annually (Li et al., 2024; Rodrigues et al., 2018).

The contemporary wound care treatments include traditional bandages, hydrogels, debridement, irrigation, antibiotics,

tissue grafting, and protein hydrolases. However, these approaches are often limited by high costs, prolonged treatment duration, potential toxicity or adverse effects, inability to adapt to dynamic wound progression, and limited applicability for wounds located in joint areas (Ahmad and Hafeez, 2023; Bhuhani et al., 2021; Noviza et al., 2026). On the other hand, conventional therapies mainly serve as protective barriers against infection and provide limited support for actively promoting wound repair (Li et al., 2024; Yang et al., 2023). Consequently, ongoing research emphasizes the development of topical formulations that provide antimicrobial protection, permit gas exchange, reduce fluid loss, support cellular proliferation, absorb wound exudates, and are biodegradable, hemostatic, affordable, and biocompatible to minimize secondary infections (da Silva et al., 2021; Yang et al., 2023).

Natural products have emerged as promising candidates for wound therapy due to their favorable safety profiles, low

cost, and high availability (Moses et al., 2023). Propolis, a resinous substance produced by bees from plant exudates, has been utilized in traditional medicine for its antibacterial and antifungal properties (Grinn-Gofroń et al., 2025), making it suitable for wound and skin dressing applications. In addition, it has been shown that propolis reduces scar formation and healing time, enhances wound contraction, and accelerates tissue repair (Abdellatif et al., 2021; Yang et al., 2023). In vitro studies have demonstrated that propolis extract (PE) promotes fibroblast migration and accelerates wound closure (M. Afonso et al., 2020). Furthermore, propolis demonstrates potent antioxidant and anti-inflammatory effects derived from its abundant phenolic constituents, such as terpenes, flavonoids (e.g., galangin), hydroxycinnamic acids (e.g., caffeic acid), and esters, which are especially beneficial in chronic wounds where sustained inflammation and oxidative stress hinder the healing process (Abdellatif et al., 2021; Conceição et al., 2022; Gal et al., 2020; M. Afonso et al., 2020).

Despite its well-documented wound-healing potential, the therapeutic efficacy of propolis is limited by the poor aqueous solubility, chemical instability, and low bioavailability of several bioactive constituents, including caffeic acid phenethyl ester (CAPE), flavonoids, and phenolic acids (Karakas et al., 2023; Stojko et al., 2021; Yang et al., 2023, 2022). To overcome these limitations, numerous topical delivery systems have been developed, including hydrogels, electrospun nanofibers, nanoemulsions, liposomes, solid lipid nanoparticles, and nanostructured lipid carriers (da Silva et al., 2021; Li et al., 2024). These delivery systems have demonstrated improved wound healing by enhancing propolis stability, prolonging drug retention, and increasing antimicrobial efficacy. Hydrogels provide a moist wound environment but often exhibit rapid drug release and limited loading of lipophilic constituents (Hemati et al., 2023). Nanoemulsions improve the solubility and skin penetration of hydrophobic compounds but may suffer from physical instability during storage (Wang et al., 2023). Electrospun nanofibers can mimic the extracellular matrix and promote skin regeneration, although their fabrication is relatively complex (Homaeigohar and Boccaccini, 2025; Vijayakumar and Rathinam, 2026). Lipid nanoparticles enhance encapsulation efficiency and controlled release but may experience drug expulsion during long-term storage (Munir et al., 2024). Consequently, there remains a need for alternative delivery systems that combine structural stability, sustained drug release, high loading capacity for chemically diverse phytochemicals, and favorable interaction with the skin barrier. Lipid-based liquid crystalline systems possess several of these characteristics and therefore represent an attractive strategy for topical propolis delivery.

Compared with conventional topical carriers, lipid-based liquid crystalline (LLC) formulations possess a highly ordered internal nanostructure capable of simultaneously incorporating hydrophilic and lipophilic compounds and have been reported to provide sustained drug release, high bioadhesion, structural similarity to the stratum corneum, and excellent

water-retention capacity (Adwan et al., 2024; Baldha et al., 2025; Bouwstra et al., 2021; Chen et al., 2020; Silvestrini et al., 2020). These properties make LLCs attractive carriers for topical wound therapy, where prolonged residence time and maintenance of a moist wound environment are desirable. Although LLC systems have been investigated as topical carriers for various bioactive compounds, their application for propolis-based wound therapy remains limited. In particular, studies integrating formulation optimization, structural characterization, in vitro drug-release behavior, and in vivo wound-healing evaluation within a single liquid crystalline propolis system are scarce. Therefore, further investigation is needed to establish the potential of LLC as a carrier for propolis in wound management.

In the present study, a lipid-based liquid crystalline cream containing propolis extract was developed and optimized for topical wound-healing application. The formulation was characterized using polarized light microscopy, differential scanning calorimetry, and X-ray diffraction, followed by physicochemical evaluation and in vitro release studies. The wound-healing potential was subsequently assessed using a rabbit incisional wound model. LC-MS/MS analysis was performed to characterize the principal bioactive constituents of the propolis extract and facilitate discussion of the observed biological activity. To address this research gap, the present study systematically developed and evaluated a lipid-based liquid crystalline propolis cream through physicochemical characterization, structural analysis, in vitro release testing, and in vivo wound-healing assessment.

2. EXPERIMENTAL SECTION

2.1 Materials

Stingless bee raw propolis was kindly donated from PT. Suhita Lebah Indonesia, Bandar Lampung, Indonesia. Materials used for formulation such as aquadest, liquid paraffin, propylene glycol, cetostearyl alcohol, PEG40, propyl paraben, methyl paraben were of pharmaceutical grade. All other chemical reagents were analytical grade. Gallic acid and Folin-Ciocalteu reagent were obtained from Sigma Aldrich. Triamcinolone cream 0.1% was purchased from PT. Interbat, Indonesia. Acetonitrile and formic acid were obtained from Merck.

2.2 Methods

2.2.1 Propolis Extraction and Characterization

Raw propolis was powdered and extracted using ethanol 70% with the ratio 1:10 (w/w) using an ultrasonic bath (Elmasonic S 180 H, Elma Schmidbauer) at 40°C for 30 minutes and periodically stirred (Yazkan and Ertop, 2025). Subsequently, the filtrate and residue were separated using Whatman 01 filter paper and the hydroalcoholic PE was concentrated on a rotary evaporator under reduced pressure at 40°C (Bankova et al., 2021). The extract was weighed and the yield was calculated according to Equation (1). The extract was finally stored in an airtight brown bottle in a refrigerator at -20°C until used.

$$\left[\text{Yield (\%)} = \frac{\text{weight of dry extract (g)}}{\text{weight of the plant (g)}} \times 100\% \right] \quad (1)$$

Phenolic compounds in propolis were analyzed by the LC-MS/MS method using a UHPLC system (ACQUITY UPLC H Class System, Waters) coupled to a tandem mass spectrometer Xevo G2-SQToF (Two Generation Stepwave Quadrupole time of flight mass spectrometry) for the quantitative determination of 53 phytochemicals. The mobile phase was water 5 mM Ammonium Formic (A) and Acetonitrile and 0.05 Formic acid (B). The reversed-phase UHPLC was equipped with an autosampler, column oven, binary pumps, and degasser with the same chromatographic conditions as previously reported (Karagecili et al., 2023).

TPC of the propolis extract was determined using the Folin-Ciocalteu method, expressed as gallic acid equivalents (GAE). Briefly, the extract was reacted with Folin-Ciocalteu reagent followed by sodium carbonate, and the mixture was incubated in the dark at room temperature. Absorbance was measured at 760 nm using a spectrometer (Thermo Scientific Genesys), and results were calculated as mg GAE per gram of extract based on a gallic acid calibration curve. The details of the methods were given in a previous study (Hernandez-Hernandez et al., 2025).

2.2.2 Antioxidant Activity Analysis

The antioxidant activity of propolis extract was evaluated using the DPPH radical scavenging assay. A 0.1 mM DPPH solution in ethanol was mixed with propolis samples at various concentrations and incubated in the dark at 30°C for 30 min. Absorbance was measured at 517 nm using a spectrometer (Thermo Scientific Genesys), and antioxidant activity was determined from the reduction in absorbance, indicating DPPH radical scavenging. Antioxidant activity was calculated using Equation (2).

$$\left[\text{Inhibition(\%)} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100\% \right] \quad (2)$$

The (IC₅₀) value, defined as the concentration required to achieve 50% inhibition, was graphically determined (Karagecili et al., 2023).

2.2.3 Base Optimization of Liquid Crystalline Containing Propolis Extract (LCPE)

The evaluation of cream bases was performed by varying concentrations of cetostearyl alcohol. Liquid crystalline preparation was initially formulated by weighing the oil phase, namely liquid paraffin, cetostearyl alcohol and paraben. They were then heated in a beaker in a water bath at 70°C until everything melted. Subsequently, the water phase, namely the mixture of

PEG-40 and distilled water, was homogenized using a homogenizer (Ultra-Turrax T18, Ika) at a speed of 5,000 rpm for 5 minutes. The oil phase whose temperature had dropped was added to the water phase and continued with homogenization again for 5 minutes at a speed of 5,000 rpm. The mixture was then ultrasonicated (Elmasonic S 180 H, Elma Schmidbauer) for 5 minutes to remove the gas in the cream. The preparation was then cooled to room temperature and stored in a closed container before use. The composition of the LCPE formula was shown in Table 1.

The optimized formula was determined based on organoleptic, homogeneity test, viscosity, spreadability, adhesion, and cycling test which met all the required criteria. The optimized liquid crystalline formulation was subsequently loaded with propolis extract at concentrations of 1%, 2.5%, and 5% prior to physicochemical characterization and in vivo evaluation. The concentrations of propolis extract (1%, 2.5%, and 5% w/w) were selected with modifications from previously reported topical propolis formulations (Batista et al., 2018; Oryan et al., 2018).

2.2.3.1 Physical Appearance, Homogeneity pH, and Viscosity Analysis

Organoleptic evaluation was performed by assessing the texture, odor, and color of the cream. Homogeneity was examined by spreading the cream on a clean glass slide, covering it with another slide, and visually observing the preparation to ensure uniformity and the absence of coarse particles (Jubaidah et al., 2023).

The pH of the formulation was measured at room temperature using a pH-meter (Milwaukee MW151 MAX). An acceptable pH range of 4–7 was targeted to match skin pH and minimize the risk of irritation (Nunes et al., 2021).

The viscosity was determined using a viscometer (IKA ROTASVIC hi-vi I) equipped with a suitable spindle at 25°C. The measurement was performed for a sample volume of 250 mL at a rotational speed of 50 rpm (Rezae et al., 2024).

2.2.3.2 Spreadability and Adhesive Test

The spreadability of LCPE was evaluated using the glass slide method. A fixed amount of cream was placed on a glass slide, covered with another glass. An additional load was then applied on the top of the glass for 2 min. After that, the diameter of the spread sample was measured, following the method described by Waghule et al. (2021).

Adhesiveness was evaluated by spreading 0.5 g of LCPE onto a glass slide and covering it with a second slide. A 250 g weight was applied for 5 min, after which the load was removed and the time required for the two slides to separate was recorded (Talat et al., 2021).

2.2.4 Characterization of liquid crystal based propolis extract (LCPE)

2.2.4.1. PLM Analysis

The crystalline structures of the LCPE were confirmed by PLM based on their phase behavior. Samples were placed between a

Table 1. Composition of Liquid Crystalline Containing Propolis Extract (LCPE)

Materials	Formula Concentration (% w/w)						Function
	1*	2*	3*	1.1**	1.2**	1.3**	
Propolis extract	0	0	0	1	2.5	5	Active ingredient
Liquid paraffin	12	12	12	12	12	12	Emollient
Propylene glycol	8	8	8	8	8	8	Humectant
Cetostearyl alcohol	5	7.5	10	5	5	5	Emulsifier
PEG 40	8	8	8	8	8	8	Emulsifier
Propyl paraben	0.10	0.10	0.10	0.10	0.10	0.10	Preservative
Methyl paraben	0.10	0.10	0.10	0.10	0.10	0.10	Preservative
Aquadest	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100	Solvent

*Base optimization
** Optimized formula used for in vivo study

glass slide and cover slip and examined using PLM. Isotropic and anisotropic characteristics were observed, and representative images were captured at room temperature (Kamali et al., 2022).

2.2.4.2. DSC Analysis

The glass transition and nematic–isotropic transition temperatures were determined by DSC using a Q5000IR calorimeter (TA Instruments, USA). To evaluate compositional interactions in LCPE, approximately 10 mg of sample was analyzed over a temperature range of 20–300 °C at a heating rate of 10 °C/min under a nitrogen atmosphere, with an empty pan serving as the reference Kumari et al. (2023).

2.2.4.3. XRD Analysis

The crystalline characteristics of the optimized formulation were evaluated using XRD, following previously described methods Narula et al. (2026); Tomar et al. (2024). Diffraction patterns of the propolis extract (PE), the optimized liquid crystalline base (LC), and the optimized liquid crystalline propolis extract (LCPE) were recorded on a Rigaku SmartLab X-ray diffractometer (Rigaku Corporation, Japan) using Cu Kα radiation (λ = 1.5406 Å) operated at 40 kV and 50 mA. Samples were scanned over a 2θ range of 5–40° with a step size of 0.01° at a scan rate of 10° min^{−1}. The resulting patterns were compared to assess changes in the crystallinity of the propolis extract following its incorporation into the liquid crystalline system.

2.2.5 In Vitro Release Studies

The in vitro release was carried out according to the previous study by Spanidi et al. (2021) with modifications (Spanidi et al., 2021). The release rate of the polyphenols of propolis was measured in a buffer solution at pH 7.4 at 37 °C. An in vitro study of the release of the polyphenols of propolis was performed by using dialysis bag.

A 1 mL of the LCPE was placed in the dialysis bag of a MWCO = 12-14 kDa. The bag was placed in buffer pH =

7.4 which was constantly stirred with a small magnetic bar and thermostated at 37 °C throughout the experiments. On specific time points (0, 5, 10, 15, 30, 60, 120, 180, and 240 minutes), samples were taken, and their polyphenol concentration was determined using the Folin–Ciocalteu method, expressed as gallic acid equivalents (GAE) (Hernandez-Hernandez et al., 2025). The buffer quantity removed is replaced by buffer pH = 7.4 at temperature of 37 °C to maintain the sink conditions (Gorantla et al., 2022; Spanidi et al., 2021).

The release data were subjected to analysis to find the best fit by a particular kinetic model. The Korsmeyer and Peppas model was used to describe drug release (Al Saqr et al., 2022).

$$\frac{M_t}{M_\infty} = k t^n$$

where Mt/M1 was the fraction of drug released at time t, k is the kinetic constant of the system, and n is the release exponent. The n value was used to differentiate between different release mechanisms as follows: Fickian diffusion (Higuchi release) for n ¼ 0.5, non-Fickian release for 0.5 < n < 1, zero-order release (case II transport) for n ¼ 1, and super case II transport for n > 1. Other models were analyzed to determine the best fit, like zero order, first order, and Higuchi model. Microsoft Excel 2010 and Graphpad prism (Graphpad Software, La Jolla, CA) was used to perform the curve fitting. The model with the highest R2 was considered the best fitting model (Al Saqr et al., 2022).

2.2.6 Stability Test

The three cream formulations underwent a cycling test involving alternating storage at 40 ± 2 °C and 4 ± 2 °C for 24 h each, with one cycle consisting of both conditions. The test was conducted over 12 days (six cycles), and organoleptic characteristics, which are color, odor, texture, and phase separation, were assessed before and after the procedure. Additionally, LCPE was stored at room temperature for six months and subsequently centrifuged at 3000 rpm for 30 min. Stability

was assessed based on any changes in color, odor, or phase separation (Sobharaksha et al., 2024).

2.2.7 Wound Healing Assay

Healthy male New Zealand white rabbits (*Oryctolagus cuniculus*), aged 3 months and weighing 1.5–2.0 kg, were obtained from Karang Anyar Village, South Lampung District. A total of 15 rabbits were randomly assigned into five groups ($n = 3$ per group). The animals were housed individually and maintained on a standard diet with free access to tap water under controlled conditions of 25 °C, 55% relative humidity, and a 12 h light–dark cycle.

All the animal experiments were performed in accordance with the “Principles of laboratory animal care” from the Faculty of Health, Dian Nuswantoro University with number 002318 / Dian Nuswantoro University/2024.

2.2.7.1. Wound Creation

Wound induction was performed under topical anesthesia using lidocaine cream (Estesia cream®, Pharmacore, Indonesia). The dorsal mid thoracolumbar area was shaved, disinfected with 70% ethanol, and full-thickness skin wounds (6 mm in width, approximately 0.5 cm in depth and 1.5 cm in length) were created using surgical blades and forceps. The animals were divided into five groups: Group I received no treatment (bare wound control); Group II served as the positive control (triamcinolone); Groups III, IV, and V were LCPE 1%, LCPE 2.5%, and LCPE 5.0%, respectively. Wounds were covered with standard surgical dressings, which were inspected daily and replaced as needed (Sh. Ahmed et al., 2019). No hemorrhage or abnormalities were observed during or after the procedure, and wound healing was monitored periodically throughout the study.

2.2.7.2. Wound Healing Evaluation

Wound appearance and closure were documented by daily digital photography, with images captured vertically at a fixed distance of 6 cm from the wound center. All photographs were standardized in size, and wound areas were quantified by pixel analysis using ImageJ software. Wound healing was assessed based on the reduction in wound area, expressed as percentage wound contraction, calculated as:

$$\left[\text{wound contraction}(\%) = \frac{\text{area for original wound} - \text{Area at nth day}}{\text{Area for original wound}} \times 100\% \right] \quad (3)$$

In addition, the time required for complete wound epithelialization was recorded for each treatment group (Damrongrungruang et al., 2021).

2.2.8 Data Analysis

All experiments were conducted in triplicate, and results are presented as mean $\pm$ standard deviation (SD). Data from pH, viscosity, spreadability, adhesiveness, REEDA scoring, hedonic evaluation and wound-healing percentage were analyzed using

ANOVA with Minitab 20.2 software. One-way and two-way ANOVA followed by Tukey's post hoc test was used to assess statistical significance, with $p \leq 0.05$ considered significant (Rosdianto et al., 2023).

3. RESULTS AND DISCUSSIONS

3.1 Propolis Extraction and Characterization

The produced thick propolis extract has a characteristic yellow to brownish color with a sticky texture and a distinctive propolis aroma. This finding is in line with that found by previous study (Boke Sarikahya et al., 2022). The characteristics of propolis such as color, texture and aroma are influenced by the origin, type of plant, type of bee and the processing process of the bee propolis breeder (Mulyati et al., 2020).

The yield obtained from propolis extraction was 21.21%. This yield is higher than other studies (Aboulghazi et al., 2022; Fachri et al., 2020). It can be caused by the use of ultrasonic and the suitable solvent. Nichitai et al. (2023) mentioned that sonication techniques also increased the yield of extraction results in a short time compared to maceration. Other studies also mentioned that the ultrasonic method provides optimal results in propolis extraction because it can increase the metabolite diffusion process using ultrasonic waves and keep thermolabile metabolites from being damaged (Bankova et al., 2021; Šic Žlabur et al., 2021). In addition, other studies found that 70% ethanol can extract more phenolic and flavonoid compounds than other ethanol percentages (Bankova et al., 2021; Kara et al., 2022).

Quantitative analysis showed that TPC of propolis ethanol extract was 249.6 ± 20.8 mg GAE/g. The TPC was higher than that reported in previous studies (Kurek-Górecka et al., 2025, 2022). The antioxidant activity evaluated through the DPPH radical scavenging assay was relatively weak, with an IC₅₀ value of 504 ± 12 ppm. In comparison, vitamin C exhibited a much stronger activity, with an IC₅₀ of 8.06 ± 0.67 ppm. These findings are consistent with previous reports showing that Romanian propolis demonstrated an IC₅₀ value of 585 ppm (Kurek-Górecka et al., 2025). It should be emphasized that the relationship between the chemical structure of phenolic compounds and their antioxidant activity in biological systems is highly complex. The antioxidant activity of individual phenolic compounds depends largely on their physicochemical characteristics, including lipophilicity, solubility, and its distribution between lipophilic and hydrophilic environments. As a natural product composed of multiple constituents, propolis contains phenolic compounds with varying degrees of lipophilicity, resulting in differences in antioxidant potency and mechanisms of action (Liaudanskas et al., 2021).

Analysis using LC-Q-TOF-MS/MS produced the two highest peaks with m/z 411.2 and 471.3 (Figure 1a to 1e). Peak identification was performed by literature search and showed characteristics as shown in Table 2. Mangostin and glycyrrhetic acid were identified as the predominant compounds in the propolis extract. This finding is consistent with previous reports indicating that mangostin represents one of the major

constituents of propolis in similar studies (Maroof et al., 2023; Meemongkolkiat et al., 2024; Ohta et al., 2023; Phuong et al., 2023).

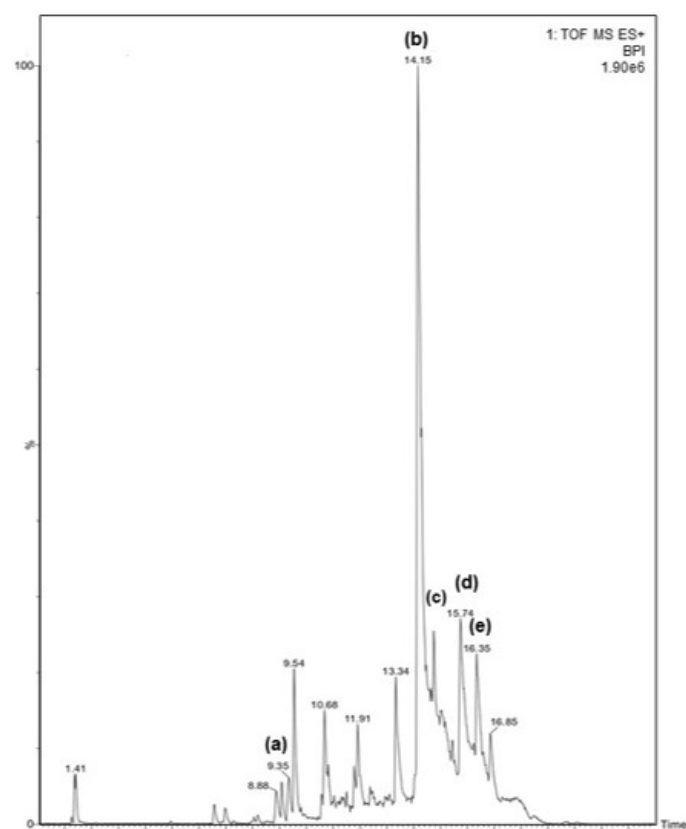


Figure 1. Base Peak Chromatogram of Propolis Extract Using LC-MS/MS, Tentative Identification of Five Major Constituents: a) Agathic Acid; b) Mangostin; c) 18- β -Glycyrrhetic Acid; d) 18- β -Glycyrrhetic Acid; and e) Betulonic Acid

3.2 Base Optimization of Liquid Crystal Based Propolis Extract (LCPE)

The objective of this study was to develop and optimize a lipid-based liquid crystalline propolis extract (LCPE) cream for topical wound-healing application and to evaluate its physicochemical characteristics, structural properties, and biological performance. The results of liquid crystal base optimization were shown in Figure 2a to 2d (F1 to F3). Liquid crystalline creams containing propolis extract were prepared using different concentrations of cetostearyl alcohol as the primary emulsifying agent. Formulation optimization was performed using an OFAT approach to evaluate the effect of cetostearyl alcohol concentration while maintaining all other formulation variables constant (Oktosa et al., 2024). The optimized formulation exhibited appropriate pH, viscosity, spreadability, adhesion, and stability characteristics, confirming its suitability for topical application (Choi, 2025; Chow et al., 2024; Tang et al.,

2025). Although Design of Experiments (DoE) provides a more efficient statistical framework for simultaneously evaluating multiple formulation variables and their interactions (Suksaeree et al., 2026), the OFAT approach was considered appropriate for this preliminary formulation study. Future studies may employ DoE to further optimize the formulation and investigate interactions among formulation components.

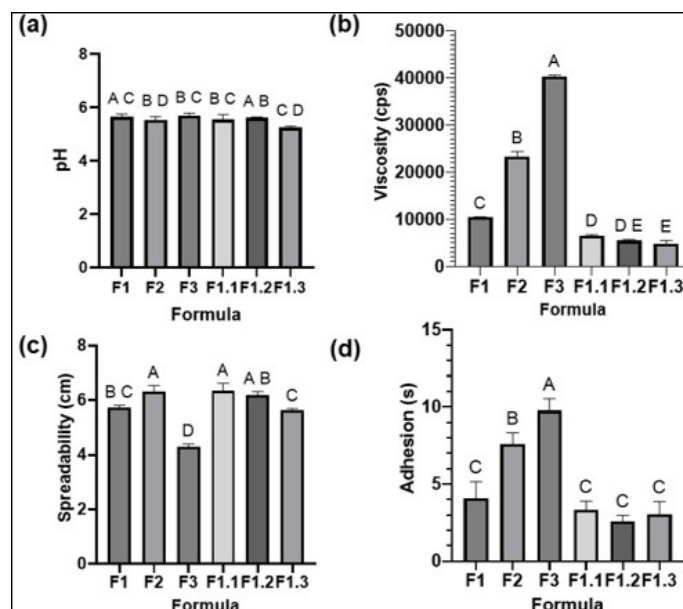


Figure 2. The Results of Liquid Crystal Base Optimization, pH (a); Viscosity (b); Spreadability (c); Adhesion (d); Significant Differences in Data Were Analyzed by ANOVA With Tukey's Pairwise Post Hoc Test at $p < 0.05$ for Means That Do Not Share a Letter

3.2.1 Physical Examination

Visual observation revealed that all cream formulations displayed a consistent white appearance, with no visible granules or phase separation when applied to a glass slide, indicating uniform homogeneity across all samples.

The pH of the liquid crystal preparations is presented in Figure 2a. The liquid crystal formulations (F1–F3) exhibited pH values ranging from 5.4 to 5.8. An increase in cetostearyl alcohol concentration did not significantly affect the pH ($p > 0.05$). Likewise, the incorporation of propolis extract did not significantly alter the pH ($p < 0.05$), as shown in Figure 2a (F1.1–F1.3). All the formulations complied with the acceptable pH range for normal skin (pH 4–7) (Choi, 2025; Tang et al., 2025). Preparations with inappropriate pH levels may elevate the risk of skin irritation or infection (Choi, 2025). Overall, the liquid crystalline base formulations showed physiologically compatible pH values, supporting their safe use for extended topical application without inducing irritation.

The viscosity data are presented in Figure 2b. The formulations (F1–F3) exhibited viscosity values ranging from 10402

to 40742 cps. Increasing the cetostearyl alcohol concentration significantly increased viscosity ($p < 0.05$), reflecting its role in thickening the cream. Higher viscosity can enhance emulsion stability by slowing the movement and diffusion of the dispersed phase within the system (Simões et al., 2019). These results are consistent with the established function of cetostearyl alcohol as a stiffening agent in cream formulations (Fauzee and Walker, 2020).

3.2.2 Spreadability

The spreadability results are presented in Figure 2c. The data indicate that increasing the concentration of cetostearyl alcohol led to a significant reduction in cream spreadability ($p < 0.05$), with values ranging from 4.11 cm to 6.48 cm. This decline in spreadability may be attributed to the increased thickness of the semisolid system, where enhanced particle-particle interactions limit molecular mobility and consequently reduce the cream's ability to spread (Wu and Zhao, 2023). Evaluation of spreadability reflects the formulation's capacity to distribute evenly across the skin surface, which may influence drug bioavailability and enhance consumer acceptance of the product (Kulawik-Pióro et al., 2019).

3.2.3 Adhesive Test

Adhesion testing serves to evaluate the formulation's capacity to adhere to the skin surface following application. A lower adhesion is considered favorable, as it may enhance user acceptability by providing a more comfortable application experience (Kulawik-Pióro et al., 2019). However, the adhesive characteristics of the base also play an important role in determining formulation effectiveness, since extended contact between the preparation and the skin may enhance therapeutic efficacy (Wu and Zhao, 2023). Therefore, sufficient adhesion is necessary to achieve optimal performance. Among the three formulations (F1–F3), increasing the concentration of cetostearyl alcohol resulted in a significant enhancement of adhesion time ($p < 0.05$), with values ranging from 1.25 to 9.53 seconds (Figure 2d).

3.2.4 Determination of Optimized Base

The evaluation of cream bases with varying concentrations of cetostearyl alcohol indicated that F1 exhibited the most favorable physical appearance, viscosity, spreadability, and adhesiveness (Choi, 2025; Chow et al., 2024; Tang et al., 2025). Therefore, F1 was selected as the optimal base for further formulation with propolis ethanol extract. The optimized formulation was subsequently prepared by incorporating propolis extract at concentrations of 1%, 2.5%, and 5% (F1.1 to F1.3 in Figure 2), followed by characterization and in vivo studies.

3.3 Characterization of The Optimized Formula

3.3.1 Physical Properties

Organoleptic assessment of the formulated preparations demonstrated that the incorporation of propolis extract imparted a pale yellow hue and a characteristic propolis scent. Notably, an

increase in the concentration of propolis extract corresponded to a more pronounced yellow coloration. The results of pH, viscosity, spreadability and adhesive test were shown in Figure 2a to 2d (F1.1 to F1.3).

The addition of the extract caused a significant decrease in the pH value ($p < 0.05$). It can be seen that F1.3, on LCPE with 5% ethanol extract had the lowest pH (5.25 ± 0.040). However, all formulations remained within the acceptable pH range for topical preparations.

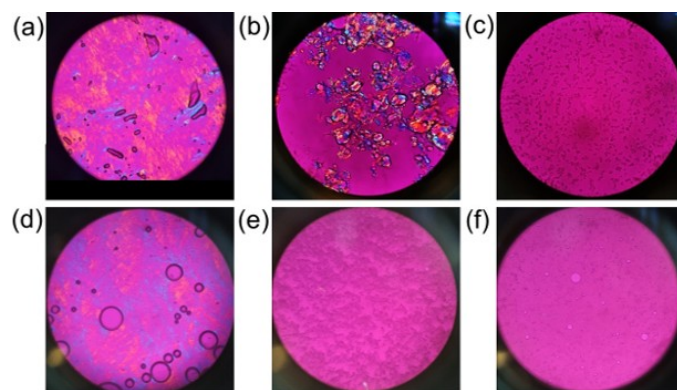


Figure 3. Polarized Light Microscopy Photograph of LLC Cream Optimized Formula, (a) Without Propolis Extract; (b) With Propolis Extract; (c) Without Cetostearyl Alcohol; (d) Without Propylene Glycol; (e) Without Cetostearyl Alcohol and Propylene Glycol; (f) Without Propolis, Cetostearyl Alcohol, and Propylene Glycol. Images Were Captured Using a Red Filter to Prevent Photoinitiation of Polymerization

The viscosity value of the propolis ethanol extract cream formulation also decreased significantly as the extract concentration increased ($p < 0.05$). Furthermore, a reduction in both spreadability and adhesiveness was observed; however, these changes were not statistically significant. This indicates that the base could maintain its consistency upon the incorporation of propolis extract.

3.3.2 PLM Analysis

PLM was used to confirm the formation of the liquid crystalline phase. Lyotropic liquid crystals that are anisotropic and rotate polarized light result in various patterns of colors and texture (Pieprzyk et al., 2022; Sobharaksha et al., 2024). PLM results were shown in Figure ??a to ??f. PLM image revealed birefringent anisotropic structures characteristic of liquid crystalline mesophases (Figure ??a), while the altered birefringence pattern following extract incorporation (Figure ??b) suggests interaction between propolis bioactive compounds and the internal lipid organization without disrupting the anisotropic mesophase. Figure ??a and ??c indicated that cetostearyl alcohol contributed to the formation of lamellar liquid crystals. The photomicrographs also showed that the structure of the LC mesophases was altered by propolis loading (Figure ??b).

Table 2. Tentative Identification of Major Constituents of Propolis Extract Based on LC–MS/MS Analysis

Rt	Measured m/z	i-FIT	i-FIT Norm	Fit conf%	Compound Name	Reference
8.88	332.1660	183.5	0.344	70.90	Agathic acid	(Paz et al., 2025)
14.15	411.1791	527.8	0.039	95.14	Mangostin	(Meemongkolkiat et al., 2024)
14.72	471.3476	258.2	2.140	83.36	18- β -Glycyrrhetic acid	(Paz et al., 2025; Pratami et al., 2024)
15.74	471.3479	310.5	0.194	82.37	18- β -Glycyrrhetic acid	(Paz et al., 2025; Pratami et al., 2024)
16.35	455.3530	257.2	0.368	69.24	Betulonic acid	(Afata and Dekebo, 2023; Alenezi et al., 2022)

Table 3. Drug Release Kinetics of LCPE and PE

Formulation	Zero-order (R2)	First-order (R2)	Higuchi order (R2)	Korsmeyer-Peppas (R2)	n
LCPE	0.974	0.979	0.982	0.993	0.660
PE (propolis extract)	0.974	0.967	0.992	0.980	0.859

3.3.3 DSC Analysis

Figure 4 shows the DSC thermograms of propolis extract, cetostearyl alcohol, propylene glycol, liquid crystal base, and propolis extract loaded liquid crystal. The propolis extract exhibited endothermic peaks between 145 and 200 °C, which are likely associated with the melting of phenolic constituents, consistent with previous reports (do Nascimento et al., 2019, 2016). The physical mixture showed an endothermic event between 25 and 105 °C attributed to polymer dehydration, followed by an exothermic decomposition peak around 300°C, indicating the absence of extract microencapsulation. In contrast, the liquid crystal base and LCPE displayed similar thermograms, characterized by initial endothermic events between 25 and 120 °C corresponding to water loss. The similarity between the LCPE and placebo thermograms, along with the disappearance of extract-specific peaks, suggested successful incorporation of the extract into the liquid crystalline system and confirms thermal compatibility (do Nascimento et al., 2019).

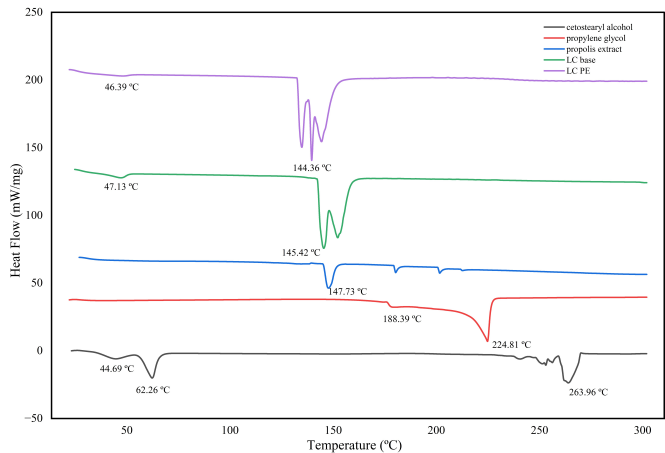


Figure 4. DSC Curves of Cetostearyl Alcohol, Propylene Glycol, Propolis Extract, LC Cream Base, and LC Cream Loaded Propolis Extract

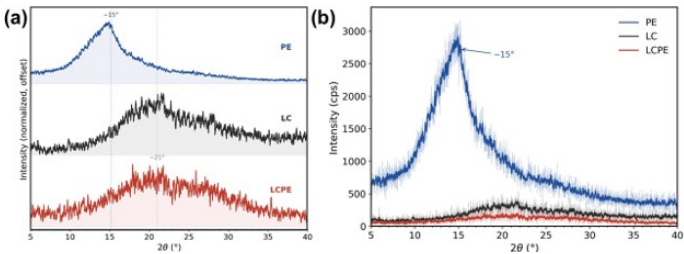


Figure 5. Stacked XRD Patterns (Normalized, Offset) (a) and Overlaid (b) of PE, LC, and LCPE; PE = Propolis Extract; LC = Liquid Crystalline; LCPE = Liquid Crystal Containing Propolis Extract

3.3.4 XRD Analysis

XRD patterns of the propolis extract (PE), liquid crystal base (LC), and liquid crystalline propolis extract (LCPE) are presented in Figure 5a and 5b. The PE diffractogram exhibited a distinct diffraction peak at approximately $2\theta = 15^\circ$, together with a broad halo over a wider angular range, indicating that the extract contained both crystalline and amorphous constituents. In contrast, the LC base displayed only broad diffuse diffraction halos without sharp peaks, suggesting that the lipid matrix predominantly existed in an amorphous or poorly crystalline state (Figure 5a and 5b). After incorporation of propolis into the liquid crystalline system, the characteristic diffraction peak of PE disappeared, and the LCPE diffractogram closely resembled that of the blank LC formulation (Figure 5a and 5b). This disappearance indicates that the propolis extract was molecularly dispersed within the liquid crystalline matrix and did not recrystallize upon incorporation. Together with the PLM and DSC results, these findings support the formation of a homogeneous liquid crystalline system in which the extract is uniformly distributed at the molecular level. The absence of newly generated diffraction peaks further suggests that no additional crystalline phase was formed during formulation, supporting the compatibility of propolis with the liquid crystalline carrier. Similar reductions in crystallinity following incorporation into lipid-

based delivery systems have been associated with improved physical stability and enhanced availability of poorly soluble bioactive compounds. This result showed a similar pattern to other study (Usapkar et al., 2024). Nevertheless, because XRD primarily provides information on crystallinity, complementary techniques such as small-angle X-ray scattering (SAXS) would be required to determine the detailed internal mesophase structure of the liquid crystalline system.

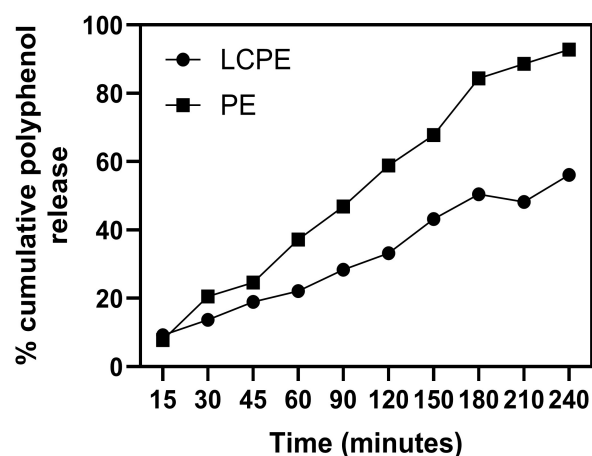


Figure 6. Polyphenol Release Profiles From LCPE in PBS (pH 7.4). Each Value Represents the Mean $\pm$ S.D (N = 3)

3.4 In Vitro Release Studies

The drug release profiles of polyphenol of developed LCPE and free drug (propolis extract, PE) data are depicted in Figure 6. PE dispersion showed a rapid release of 90 % TPC in 240 minutes (4 h) while the LCPE showed a sustained release profile with a cumulative polyphenol release around 56.14% over 240 minutes of study. The controlled drug release of TPC from LCPE was obtained due to the unique structural features of the LCPE, including aqueous and lipid channels. The observed controlled drug release pattern can be attributed to active ingredients dissolved in liquid lipid, and also the strong interaction of lipid with surfactants (Gorantla et al., 2022). The slower release of polyphenols is supposed to be caused by PE being trapped in the LC (Tomar et al., 2024).

Different mathematical models were fitted with in-vitro release data, and the best model was selected based on the highest R² value. Korsmeyer-Peppas model with R² of 0.993 was the best-fit model for polyphenol release from LCPE (Table 3). The results observed were found similar to the previously reported studies (Gorantla et al., 2022; Kouhjani et al., 2023; Rapalli et al., 2021). The value of n was found 0.660, which $1 > n > 0.50$ indicating non-Fickian or anomalous diffusion mechanism (Rapalli et al., 2021).

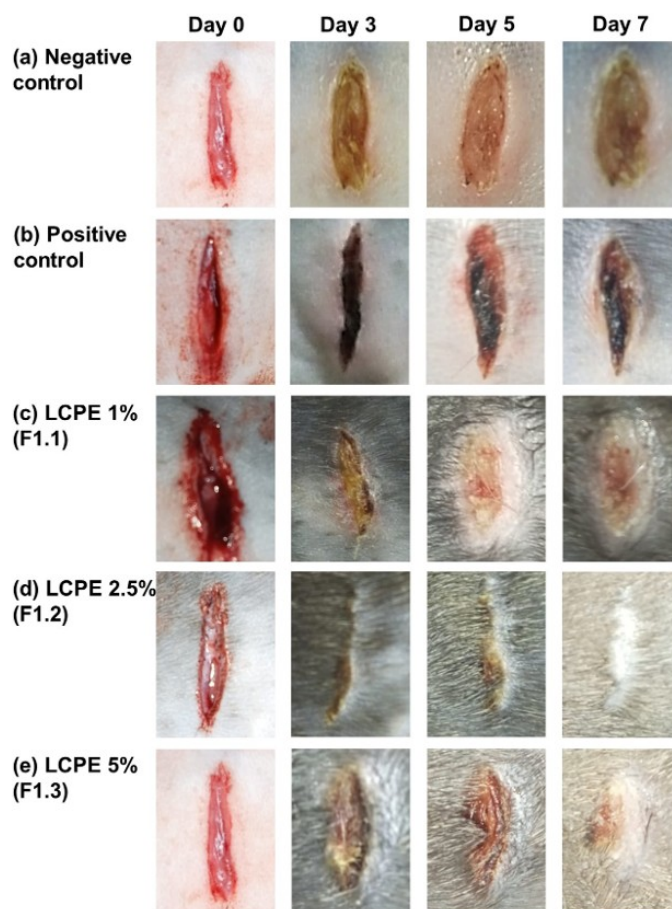


Figure 7. Macroscopic Observations of Skin Recovery in Different Groups With Time, a) Negative Control; b) Positive Control; c) LCPE 1%; d) LCPE 2.5%; e) LCPE 5%; LCPE = Liquid Crystal Contraining Propolis Extract

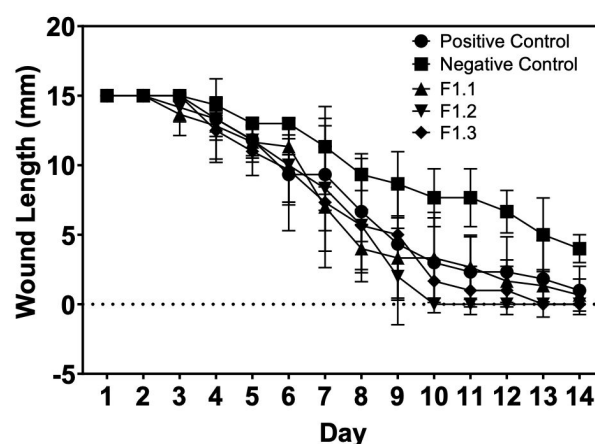


Figure 8. Average of REEDA Value in Each Group for 14 Days

3.5 Stability Test

The stability evaluation of the cream formulations in this study was conducted using a six-cycle cycling test, during which pa-

Table 4. Comparison of the Developed Liquid Crystalline Propolis Extract (LCPE) Cream With Previously Reported Propolis-Loaded Topical Delivery Systems for Wound Healing

Delivery system / formulation	Propolis concentration	Biological evaluation	Main findings & limitations	Reference
O/W nanoemulsion, propolis + tea tree oil ± clindamycin HCl	0.4% propolis (F1–F5; F5 = propolis-free)	In vitro cytotoxicity/scratch assay; in vivo rat excisional wound, histopathology	Findings: propolis (not clindamycin) drove in vitro healing; TTO gave antimicrobial effect in vivo; propolis-free NE showed no benefit; drug-loaded NE gave comparable wound-healing to drug-free. Limitations: very low viscosity limits gel incorporation; short-term study only; no comparative in vivo dosing beyond single time point.	(Abdellatif et al., 2021)
Propolis + tea tree oil + jojoba oil nanoemulgel	2.5%, 5%, 10% (optimal 5%, F-PN2)	In vitro DPPH/anti-inflammatory/MTT; in vivo rat excisional wound (MDA, SOD, TNF- α , histology)	Findings: 94.8% DPPH scavenging; faster closure, $\uparrow$ SOD, $\downarrow$ MDA/TNF- α , better re-epithelialization vs controls. Limitations: cytotoxic at higher concentrations; no chronic/infected-wound comparison.	(Saad et al., 2026)
NLC and microemulsion (ME) loaded with Brazilian red propolis (BRP)	5–10 mg/mL	In vivo rat excisional wound (13 groups, n=5); CFU counts; genotoxicity; hepato/nephrotoxicity	Findings: ME-BRP5 fastest closure; ~94% closure by day 21; no genotoxicity/organ toxicity detected. Limitations: NLC underperformed ME; no histological/mechanistic data; small groups (n=5).	(Campos et al., 2026)
Propolis solid dispersion (1:2 with Kollisolv PEG-8000) anti-inflammatory cream	2%	In vivo carrageenan paw-edema (rat) vs diclofenac gel; TNF- α ELISA; histopathology	Findings: 340-fold solubility increase; comparable anti-inflammatory efficacy to diclofenac; TNF- α reduced 276→186 pg/mL. Limitations: small cohort (n=4/group); single-dose/short-duration; no infected/chronic-wound model.	(Ratnaparkhi and Kshirsagar, 2026)
Propolis transfersome gel co-loaded with aloe vera and gotu kola	300 μ L extract + 2 mL aloe + 1.5 mL gotu kola	In vivo alloxan-induced diabetic-rat wound (21 days) vs Cutimed positive control	Findings: complete healing (0 cm) by day 12, comparable to positive control, significantly better than base /negative controls. Limitations: ζ below $\pm$ 30 mV (limited colloidal stability); small animal groups.	(Aji et al., 2024)
Collagen-hydrolysate sponge with honey-propolis wax (HPW)	28 mg/mL	In vitro HaCaT scratch/invasion; in vivo mouse excisional wound; ELISA/qPCR/Western blot (VEGF, EGF, TGF, TNF- α)	Findings: sustained release (30% at 24 h, 78% at 60 h); ~50% contraction by day 10; upregulated VEGF/EGF/TGF- β , reduced TNF- α /IL-1 β . Limitations: lower extraction yield than literature; no infected-wound/antibacterial testing.	(Ibrahim et al., 2022)
Pullulan/ PVA hydrogel loaded with Brazilian propolis ethanol extract (EEP/PS)	1.5%, 3% w/w (EEP1.5%/PS, EEP3%/PS)	In vitro: cytotoxicity (CCK-8, L929/ HUVEC/ RAW264.7), HUVEC migration under H ₂ O ₂ oxidative stress, antibacterial (E. coli, S. aureus), hemolysis/ hemostasis (rat liver model), DPPH antioxidant, qPCR/ ELISA (TNF- α , IL-6, IL-1 β); in vivo STZ-induced diabetic-rat excisional wound (n=6/ group, 12 days), H&E/ Masson histology, IHC (TNF- α , CD31), organ histopathology + blood biochemistry	Findings: EEP3%/PS gave best swelling/ hemostasis (blood-clotting index 9.98% vs. 91.5% gauze; hemostasis 100 s vs. >180 s gauze); DPPH scavenging 55%; suppressed TNF- α /IL-6/ IL-1 β ; wounds nearly fully healed by day 12 with highest collagen deposition and CD31+ neovascularization; no organ toxicity or blood-chemistry abnormalities. Limitations: authors state underlying molecular mechanisms not fully elucidated; complex/ heterogeneous EEP composition complicates batch standardization; swelling/ degradation kinetics need further detailed study.	(Huo et al., 2026)
Bilayer polyurethane/ propolis (PU/EEP) membrane on a polycaprolactone/ gelatin (PCL/ Gel) electrospun nanofibrous scaffold	0.5% w/w ethanolic propolis extract (EEP) in the PU membrane	In vitro MTT (L929 fibroblasts, days 1/4/7), antibacterial inhibition-zone assay (S. aureus, E. coli, S. epidermidis, P. aeruginosa, K. pneumoniae); in vivo Wistar-rat full-thickness wound (3 groups, n=8, days 1/5/10/15), H&E and Masson's trichrome histology	Findings: PU/EEP-PCL/Gel gave fastest closure — 2.6% wound area remaining by day 15 vs. 8.5% (PU/ EEP alone) vs. 17.9% (control); clear inhibition zone against S. aureus (5.4 $\pm$ 0.3 mm), weaker against E. coli/S. epidermidis, no activity against P. aeruginosa; non-cytotoxic; more developed dermis and denser collagen than controls. Limitations: only one EEP concentration tested (no dose-response); no cytokine or molecular biomarker analysis; antibacterial effect weak/absent against some Gram-negative species.	(Eskandarinia et al., 2020a)
Lipid-based liquid crystalline cream (LCPE)	1%, 2.5%, 5% w/w	Rabbit incisional wound model (n = 15, 5 groups); wound contraction %, REEDA scoring, ephithelialization time	Findings: LCPE 2.5% gave fastest closure (complete by day 5), lowest REEDA scores, outperformed 0.1% triamcinolone; non-linear dose response (5% less effective than 2.5%). Limitations: mechanistic pathways not confirmed (no histopathology/cytokine/oxidative-stress biomarkers); small groups (n = 3).	Present study

rameters such as color, odor, texture, pH, viscosity, spreadability, and adhesiveness were monitored. Observations revealed

that all three formulations maintained consistent color, aroma, and texture, with no evidence of phase separation. Physical

instability in topical formulations is typically indicated by visual changes following stability testing, including phenomena such as creaming, flocculation, coalescence, Ostwald ripening, or alterations in viscosity (Rocha-Filho and Maruno, 2025). However, such changes were not observed in the tested samples. The unchanged appearance of all the preparations after the stability test indicated that the preparations were stable.

3.6 Wound Healing Assay

Figure 7a to 7e presents representative photographs showing the wound on the day of the incision and after 3, 5, and 7 days of healing, respectively. Macroscopic signs of inflammation, including erythema and edema, were less evident in wounds treated with LCPE (Figure 7c to 7e) than in those treated with the base cream (Figure 7a). A similar macroscopic appearance was observed in the positive control group treated with 0.1% triamcinolone cream.

The wound-healing study demonstrated that LCPE markedly promoted the repair of incisional wounds in rabbits. As illustrated in Figure 7d, LCPE 2.5% exhibited the most rapid wound closure, achieving complete healing by day 5, whereas wounds in the control group remained incompletely healed at the same time point. Notably, the wound-healing efficacy of LCPE (Figure 7c to 7e) exceeded that of the positive control formulation (Figure 7b). This result is in agreement with previous studies reporting accelerated wound healing with propolis-based dressings (Eskandarinia et al., 2020b,a). Interestingly, LCPE at a higher propolis concentration, namely 5% (Figure 7e), had slower wound-healing activity than LCPE 2.5% (Figure 7d). Rabbit skin treated with LCPE 2.5% also showed no visible signs of scarring. This outcome is comparable to previous studies, which reported wound healing and scar prevention within 6–8 days (Ciucanu et al., 2024; Ahmad and Hafeez, 2023). Increasing the concentration to 5% did not enhance healing, suggesting a non-linear dose-response relationship. This finding differs from previous reports describing a concentration-dependent wound-healing effect of propolis (Oryan et al., 2018). One possible explanation is that certain flavonoids exhibit vitamin K antagonist activity at higher concentrations, which may interfere with the early hemostatic phase of wound healing (Kolaylı et al., 2022). However, this hypothesis requires further experimental verification.

Macroscopic evaluation using the REEDA scale revealed observable differences among the treatment groups (Figure 8). Significantly lower REEDA scores ($p < 0.05$) were observed in LCPE treatment groups compared to the negative control starting from the second day of treatment. These findings indicate that LCPE improved the clinical signs associated with wound healing, as reflected by significantly lower REEDA scores compared with the negative control. Although the reduced REEDA scores are consistent with the reported anti-inflammatory activity of propolis, inflammatory biomarkers were not evaluated in the present study.

LC-MS/MS profiling confirmed the presence of mangostin among the bioactive constituents of the propolis extract. In

addition, several phenolic and flavonoid compounds were identified, many of which have previously been associated with antioxidant, antimicrobial, and anti-inflammatory activities associated with wound repair. Mangostin has been reported to suppress NF- κ B and MAPK signaling pathways, thereby reducing the production of pro-inflammatory mediators including TNF- α , IL-6, IL-1 β , and COX-2 (Li et al., 2019; Wathoni et al., 2020). These findings are supported by the relatively high total phenolic content of the extract (249.6 ± 20.8 mg GAE/g) and its measurable DPPH radical scavenging activity ($IC_{50} = 504 \pm 12$ ppm), which is comparable with previously reported values for propolis extracts (Kurek-Górecka et al., 2025). Although the antioxidant activity was lower than that of vitamin C, the extract demonstrated the capacity to neutralize free radicals in vitro, consistent with its phenolic composition (Manginstar et al., 2024; Suhandi et al., 2025).

Besides the biological activity of propolis, the liquid crystalline carrier itself likely contributed to the observed therapeutic performance. The lamellar mesophase closely resembles the lipid organization of the stratum corneum, facilitating interaction with intercellular lipids and enhancing dermal penetration and retention of incorporated bioactive compounds (Luo et al., 2023; Yue et al., 2021). The combination of these favorable physicochemical properties with the pharmacological activity of propolis likely contributed to the enhanced wound-healing performance observed for LCPE.

To place the present formulation in the context of previous studies, representative propolis-loaded topical delivery systems were selected from the literature and are summarized in Table 4. Although this comparison is not intended to represent a systematic review, it provides an overview of the current landscape of advanced propolis-based wound-healing formulations. Compared with hydrogels, nanoemulsions, nanofibers, lipid nanoparticles, and other nanostructured carriers, lipid-based liquid crystalline systems remain relatively underexplored for propolis delivery despite their favorable structural characteristics for topical administration. The present study therefore expands the currently limited body of evidence on liquid crystalline propolis formulations by integrating formulation optimization, physicochemical characterization, structural confirmation, in vitro release evaluation, LC-MS/MS phytochemical profiling, and in vivo wound-healing assessment within a single delivery platform.

In terms of biological performance, our study revealed that LCPE 2.5% achieved complete wound closure by day 5 in the rabbit incisional wound model, representing one of the shortest healing periods among the representative formulations summarized in Table 4. The EEP/PS hydrogel required 12 days for near-complete closure in a diabetic rat model (Huo et al., 2026), the PU/EEP-PCL/Gel nanofiber dressing left 2.6% of the wound area unhealed at day 15 (Eskandarinia et al., 2020a), and most other in vivo-validated systems reported healing periods ranging from 10 to 21 days. These comparisons should be interpreted cautiously because the included studies differed substantially in animal species, wound models,

wound size, treatment protocols, and healing endpoints. Furthermore, the propolis used in these studies originated from different geographical regions and botanical sources, resulting in considerable variation in phytochemical composition and the abundance of bioactive constituents, which can substantially influence biological activity and therapeutic efficacy. Consequently, the comparisons presented in Table 4 are intended to provide qualitative context rather than direct quantitative evidence of superior therapeutic efficacy.

Table 4 also illustrates the diversity of experimental approaches used to evaluate propolis-based wound dressings. Several recent studies incorporated histopathological examination, inflammatory cytokine analysis, oxidative stress biomarkers, immunohistochemistry, and molecular techniques such as qPCR, ELISA, or Western blotting to elucidate the mechanisms underlying wound repair (Huo et al., 2026; Ibrahim et al., 2022; Wafy et al., 2026).

Overall, the findings indicate that LCPE is a promising topical delivery platform that combines the pharmacological properties of propolis with the physicochemical advantages of a liquid crystalline carrier. Nevertheless, the biological evaluation was primarily based on macroscopic wound healing and clinical assessment using REEDA scoring. Consequently, the molecular mechanisms underlying the observed therapeutic effects remain to be elucidated. Future studies should incorporate histopathological evaluation, collagen deposition analysis, inflammatory and oxidative stress biomarkers, and immunohistochemical assessment of tissue regeneration and angiogenesis markers. Such investigations would provide a more comprehensive understanding of the wound-healing mechanism of LCPE while facilitating direct comparison with other advanced propolis-based topical delivery systems. In contrast, the present study primarily focused on formulation development and therapeutic evaluation, demonstrating that LCPE possesses favorable physicochemical characteristics and effectively accelerates wound healing in a rabbit incisional wound model.

4. CONCLUSIONS

This study demonstrates that the developed liquid crystalline propolis extract (LCPE) cream is a stable and skin-compatible topical formulation with promising wound-healing potential. The optimized formulation containing 2.5% propolis exhibited favorable physicochemical characteristics, sustained in vitro release, accelerated wound closure, and improved REEDA scores compared with the control groups. These findings demonstrate that a lipid-based liquid crystalline system is a promising carrier for topical propolis delivery and, together with the in vitro antioxidant activity of the propolis extract, support the potential of LCPE as a topical delivery system for wound healing. The molecular mechanisms responsible for the observed biological effects remain to be elucidated through future histopathological, biochemical, and molecular investigations.

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