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Research Article

Comparative Study of Ethanolic and Propylene Glycol Extraction of Hemp Leaf Extracts: Phytochemical Analysis and Skincare Formulation Development

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ABSTRACT

This study investigated chemical profiles, bioactivity, and formulation potential of *Cannabis sativa* L. (hemp) leaf extracts for skincare applications. Hemp leaves were extracted using ethanol (EH) and propylene glycol (PGH), and the extracts were analyzed for phytochemical content, antioxidant activity, and astringent properties. Chemical analysis using liquid chromatography coupled with triple quadrupole mass spectrometry revealed that both extracts contained diverse phytochemicals, with cannabinoids as the predominant compounds. Ethanolic extraction demonstrated superior efficiency, yielding significantly higher concentrations of cannabidiol (CBD) at 224.90 ± 1.42 μg/mL higher than the propylene glycol extraction which has CBD at 0.83 ± 0.00 μg/mL. Bioactivity assays confirmed that EH exhibited robust antioxidant activity with IC_{50} values of 258.06 ± 34.38 μg/mL for H_2O_2 scavenging and 315.38 ± 50.10 μg/mL for DPPH scavenging, while PGH demonstrated mild astringent activity (7.11 ± 1.18%) suitable for sensitive skin applications. Despite lower phytochemical yields, PGH demonstrated superior integration into water-based skincare systems, maintaining optimal viscosity, pH compatibility, and homogeneity better than EH. Stability challenges were identified during heating–cooling cycle cycling, which triggered CBD degradation and color shifts, although the formulations maintained structural integrity without phase separation under heat-cool testing. These findings establish PGH offers superior practical advantages for cosmetic stability and formulation compatibility. Furthermore, the identification of mild astringent properties in PGH provides a novel functional justification for its use in specialized sensitive skincare, bridging the gap between raw phytochemical efficacy and industrial product viability.

Keywords: *Cannabis sativa* L., LC-QQQ, Formulation stability, Extraction efficiency, Astringent properties

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Introduction

Hemp or *Cannabis sativa* L. belongs to the Cannabaceae family and is distinguished by its richness in the cannabinoid compound group (Figure 1) [1], particularly its high abundance of cannabidiol (CBD) and a low amount of tetrahydrocannabinol (THC) compared to marijuana. CBD is the major phyto- cannabinoid, a non- psychoactive compound known for its various beneficial pharmacological effects [2]. The hemp plant is a fast-growing, short-lived species composed of several distinct anatomical components, each with unique chemical and functional properties. Among these, the flowers contain the highest concentrations of CBD and are therefore the primary source for CBD extraction and the production of CBD-enriched products. In contrast, hemp leaves exhibit comparatively lower CBD levels, which has historically limited their utilization and often resulted in their disposal as agricultural waste. However, given the substantial biomass of leaves produced per plant, their potential as an active ingredient warrants further consideration. Hemp leaves can be harvested in large quantities from a single plant, with the possibility of multiple harvests within a single growing season. These attributes contribute to reduced cultivation costs and position hemp leaves as a cost-effective and promising resource for incorporation into skincare formulations.

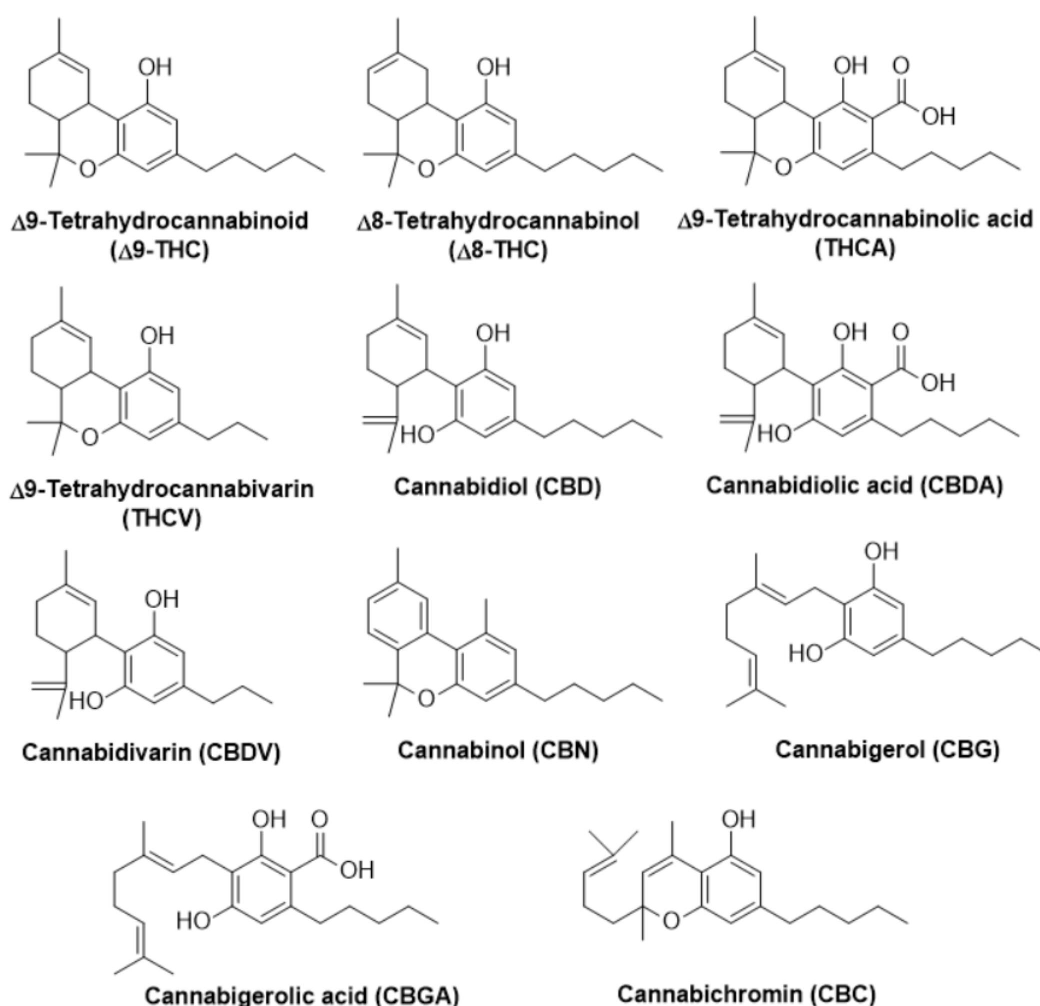

Figure 1 Cannabinoid compounds in *C. sativa* L [1].

Bioactive compounds derived from hemp exhibit a broad spectrum of skin health benefits, owing to their diverse pharmacological properties. Among these, cannabidiol (CBD) is a particularly prominent phytocannabinoid recognized for its multifunctional biological activities. Notably, CBD demonstrates strong anti-inflammatory activity, antioxidant capacity, functioning as an efficient scavenger of free radicals to mitigate oxidative stress and enhance the skin's resilience against environmental insults. Free radicals, generated through exposure to ultraviolet (UV) radiation, pollution, and psychological stress, disrupt both the structural integrity and functional balance of the skin, thereby contributing to premature aging and tissue damage [3, 4]. By neutralizing these reactive molecules, CBD provides protection against oxidative injury, supporting a healthier, more youthful skin appearance. In addition to CBD, hemp leaf extracts contain substantial levels of polyphenolic compounds, which further contribute to their bioactivity and therapeutic potential in dermatological applications.

Polyphenolic compounds demonstrate potent antioxidant activity, effectively scavenging free radicals and reducing reactive oxygen species (ROS), thereby mitigating cellular damage in the skin in a manner comparable to other established antioxidants. Beyond their antioxidative function, polyphenols present in hemp leaf extracts have been linked to skin-brightening effects, contributing to a more uniform complexion and reducing the visibility of hyperpigmentation and dark spots [5, 6]. Furthermore, their inherent astringent properties can minimize pore size and enhance overall skin texture, resulting in a smoother and more radiant appearance.

The astringent activity of plant extracts is an important property for skincare applications, particularly in formulations targeting pore refinement, excess sebum control, and skin-tightening effects. Astringency is primarily attributed to the ability of polyphenolic compounds, especially tannins, to precipitate and cross-link skin surface proteins, forming a temporary protective layer that reduces moisture loss and tightens the skin [7]. This mechanism is well established in traditional botanical medicine and is increasingly recognized in modern cosmetic science as a valuable functional property. In the context of hemp leaf extracts, tannins and flavonoids contribute to astringent activity, suggesting their potential utility in formulations designed for oily or acne-prone skin types, where pore minimization and sebum regulation are desirable outcomes [8].

Collectively, the bioactive compounds found in hemp leaf extracts offer a holistic approach to skincare, the synergistic effect of CBD and polyphenol in hemp leaf extracts make them valuable ingredients in skincare formulation, offering protection, rejuvenation, and nourishment for the skin.

Despite the promising bioactive profile of hemp leaf extracts, the selection of an appropriate extraction solvent is a critical consideration that directly influences both the phytochemical composition of the resulting extract and its compatibility with cosmetic formulation. Ethanol is among the most widely used solvents in botanical extraction due to its broad polarity range and efficiency in dissolving cannabinoids, flavonoids, and polyphenolic compounds [9]. However, the incorporation of ethanolic extracts into cosmetic formulations presents several practical challenges. Residual ethanol in finished products may cause skin irritation, dryness, and disruption of the skin barrier, particularly in sensitive skin types [10]. Furthermore, ethanol is classified as a volatile solvent, raising concerns regarding its

evaporation during manufacturing, storage stability, and compliance with cosmetic safety regulations in certain markets [10]. The relatively low boiling point and flammability of ethanol also impose additional requirements during large-scale production. These limitations have prompted interest in alternative solvents with improved cosmetic compatibility. Propylene glycol (PG) is a commonly used humectant and co-solvent in cosmetic formulations, recognized for its skin-conditioning properties and Generally Recognized As Safe (GRAS) status [11, 12]. Its higher viscosity and lower volatility compared to ethanol make it a more suitable carrier solvent in leave-on skincare products, while its polar nature allows for the extraction of a range of phytochemicals from plant material. Nevertheless, the relative extraction efficiency of propylene glycol compared to ethanol, particularly for cannabinoids and polyphenols from hemp leaves, remains underexplored. A systematic comparison of these two solvents is therefore warranted to identify the most appropriate extraction medium for developing effective and cosmetically acceptable hemp leaf formulations.

This study aimed to investigate the chemical composition and biological activities of hemp leaf extracts, with the objective of evaluating their potential as functional active ingredients in skincare formulations. This work will bridge the gap between extraction efficiency and formulation viability, extracts were prepared using ethanol and propylene glycol as comparative solvent systems, selected based on their distinct physicochemical properties and industrial relevance. The chemical profiles of the resulting extracts were characterized using liquid chromatography–triple quadrupole mass spectrometry (LC-QQQ) for the quantification of key cannabinoid compounds. Biological efficacy was subsequently assessed through the determination of total flavonoid content (TFC) and total tannin content (TTC), complemented by antioxidant activity assays including 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging and hydrogen peroxide (H_2O_2) scavenging assays, as well as astringent activity evaluation. Finally, a skincare serum incorporating hemp leaf extracts was formulated, and its physical and chemical stability was evaluated to assess suitability for cosmetic applications.

Materials and Methods

Extraction of C. sativa L. Leaves

Materials

Leaves of *C. sativa* L. were harvested on August 2023 and supplied by Rungreungkunpanit CO., LTD., Thailand and were dried under 40 °C for 48 h. 95% ethanol was purchased from RCL Labscan. Propylene glycol was purchased from Pitsanuchemicals Company Limited. Tannic acid was purchased from Tokyo Chemical Industry. Sodium nitrite and Folin-Ciocalteu's reagent were derived from LOBO CHEMIE PVT. LTD. Aluminium chloride hexahydrate and 2,2-Diphenyl-1-picrylhydrazyl were from Sigma-Aldrich company. Raw materials for cosmetic preparations were purchased from Cheme Cosmetics (Thailand).

Maceration of Hemp Leaves by Ethanol

Finely dried hemp leaves were used for the preparation of hemp leaf extracts. Ethanol was employed as the solvent in the maceration method, with a ratio of 1:10 (plant material 35 g to solvent 350 mL). The samples were soaked for 24 hours and subsequently filtered using Whatman No. 1 filter paper. The residual plant material was subjected to maceration in triplicate to ensure reproducibility. The resulting extracts were then concentrated using rotary evaporation (yielding 14.18±2.07% w/w), and the hemp leaf extracts were stored under chilled conditions until further analysis.

Solvent Extraction of Hemp Leaves by Propylene Glycol

Propylene glycol was used as the solvent in the extraction process, as it is a common ingredient in cosmetic formulations. Hemp leaves were soaked in propylene glycol at a ratio of 1:5 (plant material 50 g to solvent 250 mL) for three days. The resulting extracts were then filtered by vacuum filtration using a Büchner funnel. The hemp leaf extracts were subsequently stored under chilled conditions until further analysis.

Determination of Chemical Composition of Hemp Leaf Extracts by LC-QQQ

Hemp leaf extracts were transferred into glass vials using a syringe fitted with a 0.45 µm nylon filter prior to liquid chromatography–triple quadrupole tandem mass spectrometry (LC-QQQ) analysis. The analytical procedure was conducted following the method described in [13]. A C18 column was employed as the stationary phase, with the column temperature maintained at 35 °C. The mobile phases consisted of water (phase A) and methanol (phase B), each containing 0.1% formic acid and 2 mM ammonium formate. The flow rate was set at 0.5 mL/min, and gradient elution was applied as follows: 60 – 90% phase B from 0 to 7.00 min; 90% phase B from 7.00 to 9.00 min; 90 – 60% phase B from 9.00 to 9.50 min; and 60% phase B from 9.50 to 13.00 min. The total run time was 13 min, and the injection volume was 200 µL. The Cannabidiol (CBD) was used as a standard, using positive electrospray ionization (ESI+) with a precursor ion $[M+H]^+$ at m/z 315.10.

Evaluation of Polyphenolic Contents of Hemp Leaf Extracts

Total Flavonoid Contents

The total flavonoid content (TFC) was analyzed using aluminum chloride colorimetry following a modified method [14]. A solution of 15% w/w sodium nitrite ($NaNO_2$) was prepared, and 15 µL was added to 100 µL of diluted hemp leaf extract. The mixture was incubated for 6 min at room temperature. Subsequently, 15 µL of 15% w/w aluminum chloride ($AlCl_3$) solution was introduced, and the reaction was allowed to proceed for another 6 min at room temperature. Thereafter, 70 µL of 8% w/w sodium hydroxide (NaOH) solution was added, and the mixture was incubated in the dark for 15 min. The absorbance of the solution was measured at 510 nm, with all samples analyzed in triplicate. Quercetin (QE) was used as the standard (0.01 – 3.0 mg/mL), and the TFC of each sample

was quantified and expressed as micrograms of quercetin equivalent per milligram of extract (µg QE/mg extract).

Total Tannin Contents

The determination of total tannin content (TTC) was performed using a modified method based on a previous study employing the Folin–Ciocalteu phenol reagent [15]. In this procedure, 20 µL of diluted plant extract was mixed with 100 µL of 0.2 N Folin–Ciocalteu reagent in a 96-well plate, and the mixture was incubated for 4 min at room temperature. Subsequently, 80 µL of 7.5% sodium carbonate (Na_2CO_3) solution was added, and the reaction mixtures were incubated in the dark for 2 h. The absorbance of each sample was measured at 725 nm, with all samples analyzed in triplicate. Tannic acid was used as the standard (0.01-1.0 mg/mL), and the TTC of each sample was quantified and expressed as micrograms of tannic acid equivalent per milligram of extract (µg TAE/mg extract).

Analysis of Antioxidant Activities

DPPH Radical Scavenging Activity

DPPH or 2,2-diphenyl-1-picryhydrazyl is a stable free radical commonly used in antioxidant assay to evaluate the free radical scavenging activity of plant extracts. Hemp leaf extracts were determined the DPPH-radical scavenging activity by modified method following previous study [16]. 100 µL of Diluted hemp leaf extracts in ethanol was mixed with 100 µL of 60 µM DPPH solution in 96-well plate and incubate in the dark place for 30 mins. Then, the absorbance was measured at 517 nm. All measures were taken in triplicate. Trolox is used as a standard (0.0003-0.0075 mg/mL). DPPH activity of hemp leaf extracts was calculated with following equation:

$$\text{Inhibition}\left(\%\right) = \frac{\text{ABScontrol} - \text{ABSsample}}{\text{ABScontrol}} \times 100 \qquad \text{(Eq. 1)}$$

Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide (H_2O_2) is a reactive oxygen species used in antioxidant assay for evaluating the antioxidant activity of hemp leaf extracts by measuring their ability to scavenge H_2O_2. Antioxidant activity of hemp leaf extracts was investigated by a modified method described in the previous study [17]. A solution 40 mM H_2O_2 prepared in deionized (DI) water then mixed with 1 mL of diluted hemp leaf extract. The absorbance of the mixture was measured at 230 nm and all tests were done triplicates. Ascorbic acid was used as a standard (0.005-0.25 mg/mL) and deionized water as a control. Percent inhibition of hemp leaf extracts was calculated with equation 1 (Eq.1).

Evaluation of Astringent Activity of Hemp Leaf Extracts

Astringent activity refers to the ability of substance to cause contraction or tightening of tissue when applied topically. The *in vitro* astringent activity of hemp leaf extracts was investigated using the

following method [18]. 1 mg/mL of tannic acid was used as positive control and deionized water was used as negative control. 1 mL of 1 mg/mL hemp leaf extracts in deionized water mixed with 1 mL of 1 mg/ mL of swine hemoglobin in DI water. Solution was mixed using vortex mixer then further centrifuged at 6000 rpm at 25 °C for 30 min. Supernatant was collected and measured the absorbance at 407 nm. The astringent activity of hemp leaf extracts was calculated in the following equation 2 (Eq.2):

$$\text{Astringent activity (\%)} = \frac{\text{ABScontrol} - \text{ABSsample}}{\text{ABScontrol}} \times 100 \qquad \text{(Eq.2)}$$

Formulation of Skincare Containing Hemp Leaf Extracts

Serum is a skincare product that contains a high concentration of active ingredients formulated to target specific skin concerns. The serum was prepared by modified formulation of previous study [19]. Various concentrations of hemp leaf extracts were added into formulation (Table 1).

Table 1 Formula composition of serum containing hemp leaf extract.

Ingredients	%w/w		
	F 1	F 2	F 3
Butylene glycol	3	3	3
Propylene glycol	2	1	-
Emogel oil[1]	0.5	0.5	0.5
Disodium EDTA	0.1	0.1	0.1
Hemp leaf extract	-	1	2
Carbomer	0.4	0.4	0.4
Water	93.5	93.5	93.5
Preservative	0.5	0.5	0.5
Triethanolamine	qs to pH 5-6	qs to pH 5-6	qs to pH 5-6

[1] Emogel oil is a trade name of the mixture containing glycerin (and) glyceryl acrylate/acrylic acid copolymer (and) propylene glycol (and) PVM/MA copolymer.

Evaluation of Formulated Serum

Physical Characteristics: Viscosity, pH, and Color

Viscosity of prepared serum was evaluated using a Brookfield Cone and Plate type viscometer, model DV-III (Ametek Brookfield, USA). The pH value of the serum was measured with a pH meter. The visual perception of color was assessed using a ColorQuest XE instrument based on the CIELab system, which is commonly employed for color measurement in cosmetic products. This three-dimensional color space represents colors according to three coordinates: L*, a*, and b*. L* denotes lightness (0-100), with higher values indicating lighter colors, lower values indicating darker colors, and a midpoint of 50 representing neutral gray. The a* coordinate represents the position along the green–red axis, where positive values correspond to red hues and negative values correspond to green hues,

with 0 indicating gray. The b* coordinate represents the position along the blue–yellow axis, where positive values correspond to yellow hues and negative values correspond to blue hues, with 0 again indicating neutral gray.

Stability Evaluation

The stability of the prepared serums was evaluated using two techniques. First, centrifugation was performed at 6000 rpm and 25 °C for 30 min to observe potential phase separation. Second, an accelerated stability study was conducted through heating and cooling cycles. The serums were stored in a hot air oven at 45 °C for 24 h, followed by refrigeration at 4 °C for another 24 h, which was considered one cycle. This process was repeated for five consecutive cycles [20].

Evaluation of Chemical Stability

The formulated serum that passed the heating–cooling stability cycles was subjected to cold ethanol precipitation following the method described in [21]. Briefly, 1.0 g of serum was mixed with 9.0 g of ethanol, and the solution was homogenized using a vortex mixer. The mixture was then centrifuged at 6000 rpm and 0 °C for 30 min. The resulting supernatant was collected and filtered through a 0.45 µm nylon syringe membrane prior to further analysis by LC-QQQ, as previously described.

Statistical Analysis

All experiments were performed in triplicate using independently prepared samples (n = 3 independent experimental replicates), and results are expressed as mean ± standard deviation (SD). Statistical differences among multiple groups were assessed using one-way analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) post-hoc test for pairwise comparisons, which controls the family-wise error rate across multiple comparisons. For direct comparison between two groups, an independent samples t-test was applied. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using [SPSS version 29.0].

Results and Discussion

Qualification and Quantification of C. sativa L. Leaves Extracts

The chemical constituents of hemp leaf extracts were analyzed using liquid chromatography coupled with triple quadrupole mass spectrometry (LC-QQQ). The analysis identified cannabinoids as the primary constituents in both the ethanolic (EH) and propylene glycol (PGH) extracts, with cannabidiol (CBD) and tetrahydrocannabinol (THC) emerging as the most abundant compounds.

Quantitative analysis revealed marked differences in CBD content between the two extraction methods. At a concentration of 1.0 mg/mL, the EH extract contained 224.90±1.42µg/mL of CBD, whereas the PGH extract yielded significantly lower levels of 0.83±0.00 µg/mL. When calculated

based on the dry weight of the extract, the EH demonstrated a CBD content of 0.45±0.07% w/w. This finding aligns with reported literature values for hemp leaf material, which typically range from 0.1% to 0.5% CBD depending on the cultivar and maturity, confirming that the triplicate ethanolic maceration used in this study successfully achieved an exhaustive recovery of the available cannabinoids. However, the CBD contents by using propylene glycol has not been reported.

This disparity can be attributed to differences in solvent polarity. Ethanol, with its moderate polarity, facilitated higher cannabinoid yields and a broader cannabinoid spectrum compared to propylene glycol. Cannabinoids possess amphiphilic characteristics, comprising hydrophobic alkyl chains and polar functional groups such as hydroxyl groups, which confer partial solubility in both polar and non-polar solvents. Ethanol, as a polar solvent, interacts effectively with these functional groups through hydrogen bonding and dipole-dipole interactions, thereby enhancing cannabinoid solubility and extraction efficiency. In contrast, propylene glycol exhibits stronger polarity and is predominantly hydrophilic, resulting in weaker interactions with cannabinoids and reduced extraction efficiency. Consequently, ethanolic extraction produced higher-quality extracts with greater cannabinoid yields, while propylene glycol extraction was less effective in recovering cannabinoids from hemp leaves.

Furthermore, the observed differences in phytochemical yields are intrinsically linked to the distinct extraction protocols designed to mimic their respective industrial applications. The EH extraction was optimized for maximum phytochemical recovery through triplicate maceration and subsequent concentration via rotary evaporation, establishing a high-potency benchmark. Conversely, the PGH extraction followed a practical cosmetic manufacturing approach; a 1:5 ratio and a longer maceration period were utilized to compensate for the solvent's higher viscosity and lower diffusivity. Unlike ethanol, PGH is a non-volatile solvent intended for direct incorporation into skincare bases. Thus, the lower CBD yield in PGH represents a deliberate trade-off for "formulation-ready" convenience and structural compatibility. Consequently, while ethanol remains the superior solvent for achieving high-purity cannabinoid benchmarks, propylene glycol provides a functional, albeit less potent, alternative tailored for direct cosmetic integration.

Total Flavonoid and Tannin Content

Tannins and flavonoids were extracted from hemp leaves using ethanol and propylene glycol as solvents. The results demonstrated substantial differences in extraction efficiency between the two solvents. Total tannin content in the ethanolic extract reached 129.90±14.73µg TAE/mg extract, whereas the propylene glycol extract yielded only 0.0074±0.00 µg TAE/mg extract. Similarly, flavonoid content was markedly higher in the ethanolic extract at 65.12±9.89 µg QE/mg extract compared to 3.53±2.35 µg QE/mg extract in the propylene glycol extract. The superior extraction efficiency of ethanol can be attributed to its moderate polarity, which enables dissolution of a broader spectrum of polyphenolic compounds, including tannins and flavonoids. Additionally, ethanol penetrates plant cell walls more effectively, resulting in enhanced yields. Although propylene glycol is effective for extracting certain polar compounds, it demonstrates less efficiency than ethanol for these specific phytochemicals.

Tannins and flavonoids are highly valued in cosmetic formulations for their antioxidant properties, which protect skin from oxidative stress and environmental damage [22-24]. Tannins exhibit astringent properties that tighten and tone the skin, minimize pore appearance, and improve skin texture. Their anti-inflammatory effects make them particularly beneficial in formulations designed to soothe irritated or sensitive skin. Flavonoids, as potent antioxidants, neutralize free radicals to prevent premature aging and enhance skin radiance. Additionally, they demonstrate anti-inflammatory, antimicrobial, and UV-protective effects, establishing them as versatile ingredients in acne treatments and other therapeutic skincare products. The substantially higher flavonoid concentration in ethanolic extracts suggests that ethanol-based hemp leaf extracts may provide superior protective benefits in cosmetic applications. Ethanolic extracts, with their elevated tannin and flavonoid content, are particularly advantageous for high-performance skincare formulations. However, propylene glycol extracts retain value despite lower phytochemical concentrations, offering distinct advantages in formulations where reduced volatility and enhanced skin compatibility are priorities. Propylene glycol functions effectively as a humectant in cosmetics, promoting moisture retention and improving product texture and sensory characteristics upon application.

Antioxidant Activities of Hemp Leaf Extracts

Two bioassays were employed to assess the antioxidant activity of hemp leaf extracts: the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and the hydrogen peroxide (H_2O_2) scavenging assay. Although both methods evaluate antioxidant capacity, they target different reactive species through distinct mechanisms. The DPPH assay measures the ability of compounds to scavenge stable DPPH radicals, which exhibit a characteristic deep violet color. Antioxidant compounds in hemp leaf extracts reduce DPPH radicals, causing a color transition from violet to yellow that indicates radical scavenging activity. In contrast, the H_2O_2 assay evaluates the capacity of compounds to scavenge hydrogen peroxide, a reactive oxygen species (ROS) generated during oxidative stress. Antioxidants either directly react with H_2O_2 or catalyze their decomposition into water and oxygen, thereby reducing H_2O_2 concentration. This assay is particularly useful for evaluating compounds potential to mitigate oxidative stress-related injuries and diseases associated with hydrogen peroxide accumulation. Table 2 presents the percentage inhibition of standards and hemp leaf extracts. The ethanolic extract (EH) demonstrated superior antioxidant activity with IC_{50} values of 258.06±34.38 μg/mL for H_2O_2 scavenging and 315.38±50.10 μg/mL for DPPH scavenging, indicating stronger H_2O_2 scavenging capacity. Conversely, the propylene glycol extract (PGH) exhibited significantly lower antioxidant activity, with IC_{50} values of 3,902.57±287.35 μg/mL for DPPH scavenging and 9,974±136.39 μg/mL for H_2O_2 scavenging.

The results show that hemp leaf extracts contain diverse phytochemicals with natural antioxidant properties capable of penetrating the skin barrier to scavenge free radicals and reactive oxygen species (ROS). This antioxidant activity, confirmed through their ability to reduce DPPH radicals and decompose hydrogen peroxide, enables multiple protective mechanisms: reducing oxidative damage, defending against UV-induced oxidative stress, improving skin texture, diminishing wrinkles, promoting collagen

synthesis, and enhancing the skin's natural repair processes. The synergistic effects of the diverse antioxidant compounds present in hemp leaf extracts may amplify their overall protective capacity, providing comprehensive defense against environmental stressors and promoting skin resilience [25].

The antioxidant assays revealed a significant disparity in potency between the two solvents, with the ethanolic extract (EH) demonstrating a vastly superior capacity to neutralize both DPPH and H_2O_2 radicals compared to the propylene glycol extract (PGH). With IC_{50} values, EH serves as a high-potency antioxidant source capable of providing robust protection against oxidative stress and environmental damage. Conversely, while PGH exhibited markedly lower activity, it still maintained a measurable, mild antioxidant effect suitable for sensitive skin applications where high concentrations of active compounds might otherwise cause irritation. These findings suggest that while ethanolic extracts are ideal for intensive rejuvenation and anti-aging treatments, PGH extracts offer a practical, skin-compatible alternative for daily maintenance, thereby establishing hemp leaf extracts as versatile and valuable multi-functional ingredients for the cosmetics industry.

Table 2 Antioxidant capacity of ethanol and propylene glycol hemp leaf extracts on DPPH and H_2O_2 scavenging activity

Samples	**IC_{50}**	
	DPPH scavenging (µg/mL)	**H_2O_2 Scavenging (µg/mL)**
EH	315.38±50.10	258.06±34.38
PGH	3902.57±287.35	9974.08±136.39
Ascorbic acid	3.19±0.08	131.44±5.75
Trolox	3.10±0.05	135.60±2.11
Quercetin	2.74±0.05	71.302±1.96
Tannic acid	1.68±0.10	65.33±0.82

Note: EH: ethanolic hemp leaf extracts, PGH: propylene glycol hemp leaf extracts.

Evaluation of Astringent Activity

The astringent properties of hemp leaf extracts were investigated using the hemoglobin precipitation technique. As shown in Figure 2, tannic acid (positive control) exhibited 88.76±0.96% astringent activity, while propylene glycol hemp extract (PGH) demonstrated 7.11±1.18% astringent activity. The underlying mechanism of astringency is attributed primarily to tannins, which are polyphenolic compounds capable of cross-linking and precipitating proteins through their multiple phenolic hydroxyl groups. This protein-precipitating activity is known to be influenced by the molecular weight and structural complexity of the tannin; polymeric tannins with molecular weights exceeding approximately 2,500 Da tend to precipitate hemoglobin as efficiently as tannic acid itself, whereas lower-molecular-weight polyphenols exhibit comparatively weaker astringency [18]. The relatively moderate

tannin content detected in hemp leaf extracts likely accounts for the observed low-to-moderate astringent response [26].

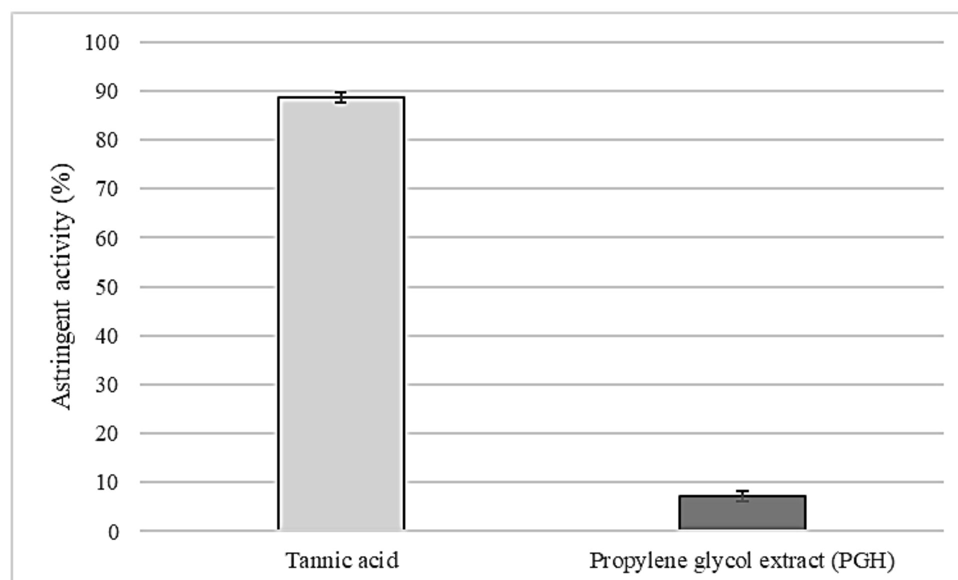

Figure 2 Astringent activity of propylene glycol extract (PGH) leaf extracts and tannic acid (standard).

Contextualizing the astringent activity of PGH within the broader literature provides a more nuanced interpretation of its practical significance. A study on green apple rind (GAR) extracts demonstrated that the 70% ethanol extract showed the highest antioxidant activity and tannin content among the extracts tested, with the ethyl acetate fraction (GAR-E) exhibiting the most notable astringent activity and inhibitory effects on 5-α reductase, a key enzyme involved in sebum regulation. The aqueous fraction of the same extract (GAR-A) showed considerably lower astringent activity than the organic solvent fractions, a pattern consistent with the present findings, where propylene glycol — a relatively polar solvent — yielded an extract with mild astringent properties. This further supports the conclusion that solvent polarity substantially influences the extraction efficiency of tannin compounds and, consequently, the astringent profile of the resulting extract [18].

A screening study of 17 local vegetables from Northern Thailand reported strong correlations between total tannin content, astringent activity, and antioxidant capacity (DPPH and ABTS radical scavenging), suggesting that tannin-rich plant extracts with measurable astringent activity are strong candidates for topical cosmetic applications including sebum regulation and pore refinement. Among the vegetables investigated in that study, *Polygonatum odoratum* demonstrated the highest astringent activity with a correspondingly high hydrogen peroxide scavenging capacity, reinforcing the dual functional value of astringent plant extracts in skincare. The astringent activity observed in PGH, while lower than that of high-tannin plant fractions, falls within a biologically meaningful range supported by reported values for edible plant extracts used in cosmetic contexts, including *Brassica alboglabra* (Chinese kale) [15].

The mechanism by which tannins exert astringent effects on the skin is well established. Tannins are phenolic compounds rich in hydroxyl residues that impart astringent properties through cross-linking with keratin proteins in the skin, creating an invisible film that can temporarily degrease the skin, tighten and tone skin texture, and minimize the appearance of enlarged pores [27]. In this regard, tannins in hemp leaf extracts function not only as antioxidants with physiological and pharmacological relevance but also as key agents responsible for reducing enlarged pore size and modulating sebum secretion, making them valuable multifunctional components in cosmetic formulations.

It has further been demonstrated that the sensory astringency of tannin-rich plant extracts is statistically correlated with their total antioxidant activity and reducing power, suggesting that the two properties are mechanistically linked through the shared polyphenolic structure responsible for both protein precipitation and free radical scavenging [28]. This correlation supports the interpretation that PGH, despite its low absolute astringent activity value, retains functional relevance in a cosmetic serum matrix, particularly given its concurrently demonstrated antioxidant capacity.

The low astringent activity of hemp leaf extracts may be regarded as a favorable characteristic rather than a limitation, particularly in the context of sensitive skin formulation. Strongly astringent agents such as alcohol and witch hazel, while effective as facial toners, can cause unwanted dryness when applied in excessive amounts. In contrast, mildly astringent plant extracts offer a gentler alternative that achieves pore-refining benefits without disrupting the skin barrier or inducing irritation. Astringent herbs draw together and constrict body tissues while reducing irritation and inflammation, creating a protective barrier that supports skin recovery without the harshness associated with more potent synthetic astringents. These properties position hemp leaf extracts as particularly well-suited for incorporation into daily leave-on skincare products, where long-term tolerability is a primary formulation consideration. However, it should be acknowledged that the low astringent concentration may prove insufficient for individuals requiring stronger pore-clearing and oil-controlling effects, and consistent, prolonged use may be necessary to achieve outcomes comparable to higher-potency astringent formulations. Nevertheless, for sensitive skin types or routine maintenance purposes, hemp leaf extracts represent a cosmetically compatible ingredient offering gentle yet effective astringent benefits.

Skincare Formulation with C. sativa L. Leaf Extracts Loaded

Hemp leaf extracts were incorporated into skincare formulations to harness their beneficial properties. Rich in cannabinoids and endowed with antioxidant activity, these extracts have the potential to regulate sebum production and promote overall skin balance. The extracts were integrated into a skincare base containing hydrating agents and emollients, with the complete formulation presented in Table 1.

The use of ethanolic extract (EH) in skincare formulations proved challenging due to its limited solubility in water-based systems, which resulted in heterogeneous mixtures. This incompatibility can be attributed to several factors. First, the complex composition of EH includes compounds that are poorly soluble in aqueous environments. Second, chemical interactions between crude EH constituents

and formulation components may generate insoluble complexes or precipitates, thereby hindering complete dissolution. Third, the relatively high concentration of EH may exceed the solubility threshold in water-based formulations, leading to precipitation. Finally, the presence of lipids and other non-aqueous components in EH further complicates solubility, as these substances are inherently incompatible with aqueous systems.

In contrast, the 0–2% w/w of propylene glycol extract (PGH) was selected for formulation development due to its superior compatibility, yielding a homogeneous final product. Hemp leaf extracts dissolved in propylene glycol (PG) readily dispersed in water-based formulations owing to PG's physicochemical properties. PG is a hygroscopic, water-soluble liquid widely used in cosmetic products as both a solvent and humectant. Its solubility characteristics enable stable solution formation across a range of concentrations. When hemp leaf extracts were dissolved in PG, they were uniformly dispersed within the solvent. Upon incorporation into the aqueous skincare base, PG facilitated homogeneous distribution of the hemp extract throughout the formulation. Figure 3 illustrates the final skincare product developed using PGH.

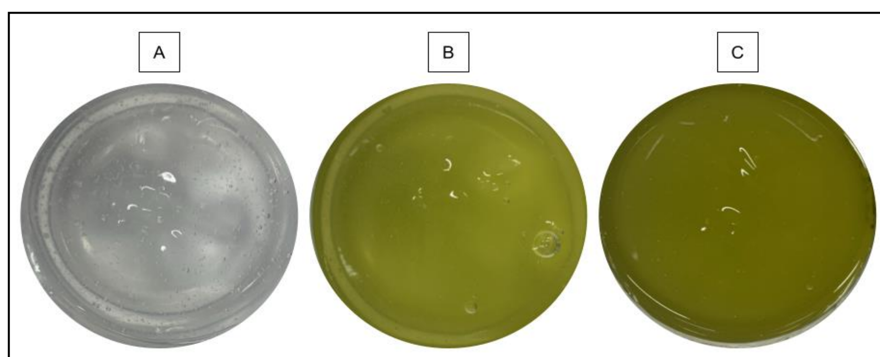

Figure 3 Physical appearance of formulated base serum (F1), serum containing hemp leaf extract at 1% w/w (F2) and 2% w/w (F3).

To evaluate the physical and chemical stability of the developed serum, the formulation was subjected to stability testing in which viscosity, pH, and color were monitored as key quality indicators. These parameters were selected based on their direct relevance to product performance, consumer acceptability, and the chemical integrity of the active constituents. The results of the stability assessment are presented in the following section. Figure 3 illustrates the final skincare product developed using PGH.

Stability of Serum Containing Hemp Leaf Extracts

Heating–cooling cycle testing is a widely recognized accelerated stability assessment method. The heating–cooling cycle provides valuable insights into the resilience of formulations when subjected to fluctuating environmental conditions, such as those encountered during storage, transportation, and consumer use. By repeatedly exposing the product to alternating high and low temperatures, this method

enables the early detection of potential instability, including phase separation, precipitation, changes in viscosity, pH drift, and color alterations.

The importance of this testing lies in its ability to simulate long-term storage conditions within a shortened timeframe, thereby predicting product shelf life and ensuring consistent quality. Moreover, heating-cooling cycle testing helps identify incompatibilities among formulation components, guiding necessary adjustments to improve product robustness. For cosmetic products, where consumer safety, efficacy, and sensory attributes are critical, such stability evaluation is essential to guarantee that the formulation maintains its intended performance and aesthetic properties throughout its lifecycle.

Viscosity, pH, and Color

The viscosity and pH of hemp leaf skincare formulations are critical parameters that profoundly influence efficacy, stability, and skin compatibility. As shown in Table 3, the formulation exhibited a viscosity ranging from 70,000 to 80,000 cPs and a pH of 5. This viscosity range produces a gel-like texture that may enhance skin barrier protection. The thick consistency creates a physical barrier on the skin's surface, locking in moisture and protecting against environmental stressors. This property is particularly beneficial for individuals with dry or sensitive skin requiring enhanced hydration and protection. Additionally, this viscosity range supports better formulation stability. The thick consistency prevents ingredient separation and maintains product homogeneity over time, ensuring that the hemp leaf skincare formulation retains its efficacy and quality throughout its shelf life while delivering consistent skincare benefits. Furthermore, the formulation's pH of 5 closely aligns with the skin's natural pH level (approximately 4.5-5.5), minimizing the risk of irritation and maintaining the skin's delicate acid mantle balance [29].

Table 3 Physical properties of base skincare and hemp leaf skincare product.

Sample	Viscosity (cPs)	%Torque	pH
F1	78533.33±230.94	98.17±0.29	5
F2	71360.00±711.06	89.20±0.89	5
F3	73946.67±333.07	92.43±0.42	5

*Viscosity is performed by Brookfield RV-2 RV Standard Spindle at 200 rpm

Color significantly influences the perception and acceptance of skincare products [30, 31]. The CIE L* a* b* color space is a widely used color model in the cosmetic industry for quantifying and describing colors based on three parameters: L* (lightness), a* (red-green axis), and b* (yellow-blue axis). In addition to individual parameter changes, the total color difference (ΔE^*) was calculated according to the equation: $\Delta E^* = \sqrt{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})}$. To provide an integrated assessment of overall color change. In this research, color measurements of skincare formulations under different conditions were performed, with results presented in Table 4.

Following heating–cooling cycling, all formulations exhibited measurable changes across the three color parameters. The L* values decreased in all samples, with the most pronounced reduction observed in F2 (ΔL* = -1.86, representing a 5.05% decrease), followed by F1 (ΔL* = -1.02, -2.65%) and F3 (ΔL* = -0.25, -0.71%), indicating a trend toward darker coloration that was most evident in formulations containing higher extract concentrations. With respect to the a* axis, F1 showed a marginal shift toward the green direction (Δa* = -0.11), whereas F2 and F3 exhibited positive Δa* values of +0.57 and +0.16, respectively, reflecting a reduction in green color intensity likely attributable to chlorophyll degradation under thermal stress. The b* values decreased across all formulations, with F3 showing the largest shift (Δb* = -2.64), followed by F2 (Δb* = -2.49) and F1 (Δb* = -0.32), indicating a reduction in yellow coloration and a shift toward a more neutral or bluish hue.

The total color difference values (ΔE*) calculated for F1, F2, and F3 were 1.07, 3.16, and 2.66, respectively. Based on established perceptibility thresholds in colorimetry, a ΔE* value below 1.0 is considered imperceptible to the human eye, values between 1.0 and 2.0 are perceptible only under close observation, and values between 2.0 and 3.5 represent a clearly perceptible difference to an average observer. Accordingly, the color change in F1 (ΔE* = 1.07) was minimal and unlikely to be discernible under normal use conditions. In contrast, F2 (ΔE* = 3.16) and F3 (ΔE* = 2.66) exceeded the threshold for perceptible color difference, suggesting that formulations containing higher concentrations of PGH are more susceptible to color alteration under thermal stress conditions. This concentration-dependent color instability is consistent with the greater abundance of thermolabile pigment compounds, particularly chlorophylls and carotenoids, present at higher extract loadings.

Temperature fluctuations and oxidation reactions profoundly affect the color of hemp leaf skincare formulations following heating–cooling testing. Temperature variations during cycling disrupt the stability and composition of pigments and other color-contributing compounds within the formulation. Elevated temperatures accelerate chemical reactions such as oxidation, which alter the formulation's color profile, while physical changes including recrystallization of excipients may further impact color perception [30, 31]. Oxidation reactions occurring upon exposure to oxygen lead to the degradation of the characteristic greenish pigments of hemp leaf formulations, generating brownish or yellowish oxidation products that contribute to the observed shifts in a* and b* values [25]. Furthermore, oxidative processes generate free radicals and reactive oxygen species that exacerbate color alterations through multiple chemical pathways, an effect that becomes increasingly pronounced at higher extract concentrations as evidenced by the larger ΔE* values recorded for F2 and F3.

Table 4 L*, a*, b* color results of hemp skincare formulated at different conditions.

Condition	Sample	L*	a*	b*
After preparation	F1	38.54±0.90	0.05±0.13	0.05±0.14
	F2	36.86±1.18	-1.26±0.16	4.90±0.23
	F3	35.09±1.08	-1.38±0.12	6.88±0.43
After heating-cooling cycles	F1	37.52±2.32	-0.06±0.16	-0.27±0.13
	F2	35.00±2.29	-0.69±0.28	2.41±0.12
	F3	34.84±2.42	-1.22±0.15	4.24±0.79

To evaluate cannabinoid content in skincare formulations, cold ethanol precipitation was employed as an extraction technique based on its ability to selectively precipitate certain components while maintaining hemp leaf extract bioactive compounds in solution. The cold ethanol precipitation process involves cooling ethanol to 0 °C and subsequently mixing it with the hemp leaf extract-containing skincare formulation. At these low temperatures, unwanted compounds such as waxes, chlorophyll, and non-polar substances precipitate, yielding a purified extract enriched in bioactive compounds including cannabinoids, terpenes, tannins, and flavonoids. Cold ethanol precipitation offers advantages of simplicity, cost-effectiveness, and scalability, making it suitable for processing large volumes of skincare formulation samples. The resulting clarified supernatant is optimized for subsequent chromatographic and mass spectrometric analysis. Table 5 summarizes the chemical stability analysis of hemp leaf extracts.

Table 5 Chemical stability of CBD content in skincare formulated.

Sample		CBD content (μg/mL)
After preparation	F2	1.60±0.02
	F3	2.83±0.01
After heating-cooling cycles	F2	0.11±0.02
	F3	0.07±0.00

Table 5 summarizes the chemical stability of CBD content in the developed skincare formulations. The initial CBD concentrations were 1.60±0.02 μg/mL and 2.83±0.01 μg/mL for F2 and F3, respectively, reflecting the concentration-dependent loading of PGH into the formulation matrix. Following heating-cooling cycling, CBD content declined markedly to 0.11±0.02 μg/mL in F2 and 0.07±0.00 μg/mL in F3, representing degradation of 93.1% and 97.5% of the initial content, respectively. These substantial losses indicate that CBD is highly susceptible to degradation under the thermal stress conditions applied, and that higher initial extract loading does not confer proportionally greater stability. Notably, F3, which contained the highest initial CBD concentration, exhibited the greatest absolute loss (2.76 μg/mL) and the lowest percentage remaining (2.5%), suggesting that

elevated cannabinoid concentrations within the formulation matrix may accelerate degradation kinetics rather than provide a protective reservoir effect.

The extensive CBD degradation observed can be attributed to multiple interconnected mechanisms. First and foremost, CBD is a thermolabile compound whose molecular integrity is highly sensitive to temperature variation. The repeated heating–cooling cycles imposed during stability testing created conditions that promoted accelerated degradation through several pathways. Oxidative degradation is considered the primary mechanism, as CBD contains a resorcinol moiety and an alkyl side chain that are inherently susceptible to attack by reactive oxygen species (ROS) and atmospheric oxygen. Under thermal stress, dissolved oxygen within the aqueous formulation matrix becomes more reactive, facilitating the conversion of CBD into oxidized derivatives such as cannabidiol hydroxyquinone and other secondary oxidation products, which are either less bioactive or entirely inactive [32].

A second critical degradation pathway is the acid- or base-catalyzed cyclization of CBD to Δ9-tetrahydrocannabinol (THC) or other cyclic cannabinoid isomers. This isomerization reaction is thermodynamically favored at elevated temperatures and can be further promoted by the pH conditions of the formulation matrix and the presence of transition metal ions from formulation excipients acting as catalysts. The extent of this conversion is of particular regulatory concern, as it may alter the legal compliance status of hemp-derived cosmetic products. Furthermore, hydrolytic degradation may occur within the aqueous serum base, wherein water molecules participate in the cleavage of ester or ether linkages within the cannabinoid structure, a process that is substantially accelerated by temperature cycling.

Beyond intrinsic CBD instability, formulation-related factors also contributed to the observed degradation. The aqueous continuous phase of the serum formulation provides a medium in which pro-oxidant reactions are more readily propagated compared to anhydrous systems. The absence of dedicated antioxidant excipients or chelating agents in the base formulation may have further exacerbated oxidative degradation. Additionally, the physical disruption caused by repeated temperature cycling, including potential microphase separation and changes in viscosity, may have altered the microenvironmental conditions surrounding CBD molecules, rendering them more accessible to degradative agents [33].

These findings highlight a critical formulation challenge: while PGH demonstrates satisfactory physical compatibility and homogeneity within the serum base, the chemical stability of its primary bioactive constituent, CBD, is severely compromised under thermal stress conditions. This represents a significant limitation for the practical application of hemp leaf extract-based skincare products and underscores the necessity of implementing protective formulation strategies. Future work should systematically investigate the incorporation of antioxidant excipients such as tocopherol (vitamin E), ascorbyl palmitate, or butylated hydroxytoluene (BHT), the use of chelating agents such as EDTA to sequester pro-oxidant metal ions, encapsulation technologies such as liposomes or cyclodextrin inclusion complexes to shield CBD from the aqueous environment, and nitrogen-purged or vacuum-sealed packaging to minimize dissolved oxygen content throughout the product shelf life.

Conclusions

This study demonstrated that *C. sativa* L. leaf extracts have meaningful potential as bioactive ingredients in skincare formulations, with solvent selection being a key determinant of both phytochemical yield and formulation viability. Ethanolic extraction yielded superior cannabinoid and polyphenol content with stronger antioxidant activity, whereas propylene glycol extraction, despite lower phytochemical concentrations, offered greater compatibility with aqueous cosmetic systems and produced homogeneous formulations with acceptable physical characteristics. The mild astringent activity of PGH further supports its suitability for sensitive skin applications. However, stability testing revealed that CBD was highly susceptible to oxidative degradation under thermal stress, representing a critical challenge for product shelf-life.

Several limitations should be acknowledged. Extraction conditions between the two solvent systems were not fully standardized, limiting the directness of phytochemical comparisons. Biological activity was assessed by *in vitro* assays only, and no long-term stability data under ICH-compliant conditions were generated.

Future work should prioritize the optimization of extraction parameters, the incorporation of protective excipients such as antioxidants and encapsulation systems to address CBD instability, and the conduct of *ex vivo* skin penetration and *in vivo* tolerability studies to support the translation of these findings into viable cosmetic applications.

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