Research Article

EXPLORING THE PHOTOPROTECTIVE POTENTIAL OF CALENDULA OFFICINALIS L. ESSENTIAL OIL IN SKINCARE

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Abstract

The study investigated the potential of Calendula officinalis L. essential oil in skincare formulations with sun protection properties. A comprehensive methodology encompassed extraction, characterization, formulation, and evaluation of the oil's efficacy. Physicochemical parameters assessment indicated favorable results, with GC-MS analysis identifying twenty-two compounds, notably 1,8 cineole and α-pinene, with antioxidant properties suggesting UVB protection. Formulation of stable oil-in-water creams containing 1-5% Calendula oil exhibited no phase separation and maintained viscosity, highlighting robust physical stability. In vitro SPF testing demonstrated significant sun protection activity, particularly in formulations with higher oil concentrations. The study supports Calendula-based formulations as promising natural alternatives for sun protection in cosmetics, offering a sustainable approach without synthetic additives.

Keywords: Herbal formulations, Suncream, Antioxidant, WRINKLES

1. Introduction

Centuries of herbal medicine and cosmetics underscore the efficacy of botanicals in skincare, particularly in combating the damaging effects of ultraviolet (UV) radiation on the skin. Herbal formulations rich in antioxidants like vitamins C and E, flavonoids, and phenolic acids offer potent protection against UV-induced skin damage. Studies demonstrate the effectiveness of plant extracts such as green tea and aloe vera in mitigating adverse reactions from UV exposure, driving the popularity of herbal sunscreens[1-16].

The UV spectrum, comprising UVC, UVB, and UVA radiation, poses significant risks to skin health, including sunburn, premature aging, and cancer development. UVB radiation, though partially absorbed by the atmosphere, causes photochemical damage to DNA and is a leading contributor to skin damage and cancer. Free radicals generated by UVB exposure trigger oxidative stress, leading to collagen breakdown, lipid peroxidation, and structural alterations in the skin[17].

Protective measures against UVB exposure include the use of broad-spectrum sunscreens, which absorb, reflect, or scatter solar rays. Higher Sun Protection Factor (SPF) values indicate superior protection against sunburn. Despite the ozone layer absorbing most UVC radiation, accidental exposure can cause corneal burns. UVA radiation induces tanning and poses risks of immune suppression, cataracts, and cancer.

UVB radiation's harmful effects extend to eye health, immune suppression, collagen breakdown, and cell death, highlighting the importance of effective photoprotection strategies. Herbal formulations, enriched with antioxidants and active sun screening agents, offer promising avenues for skin protection against UV-induced damage, paving the way for safer and more effective photoprotection options in skincare[2,18,19].

Certain herbal sunscreen agents, like rose oil, offer a plethora of benefits for the skin. They possess properties that can soothe sunburns, deeply hydrate the skin, and aid in the repair of damaged skin cells. Additionally, the antioxidants present in green tea are known for their ability to counteract the damaging effects of harmful UVB radiation on the skin. These antioxidants help protect the skin from oxidative stress caused by UV exposure, thereby reducing the risk of sun damage and promoting healthier skin[3,13].

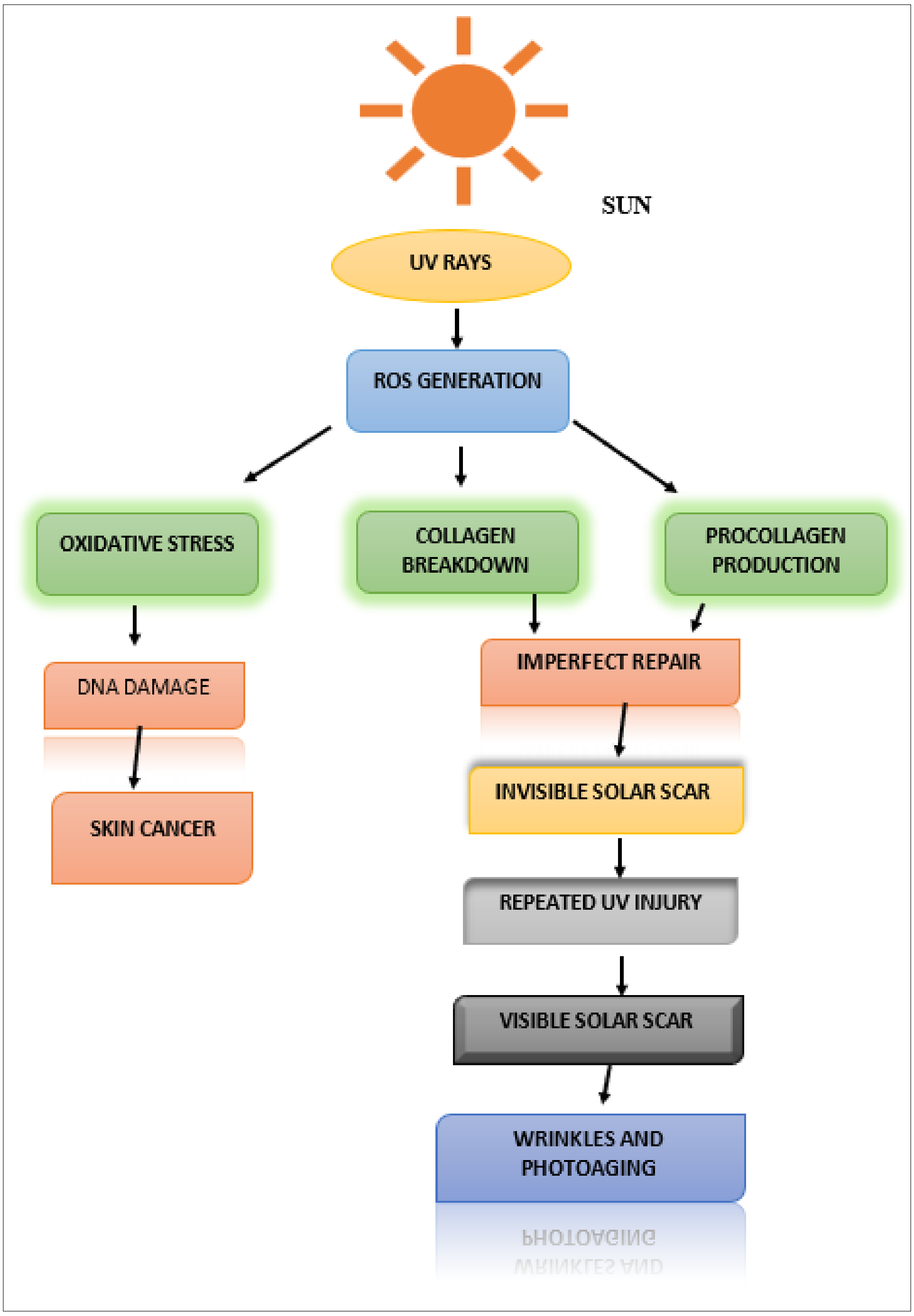

Figure 1: Effect of ROS on skin

1.1 Plant C. officinalis Flowers

Calendula officinalis L., commonly known as English Garden Marigold, is a member of the Asteraceae family prized for its cosmetic and therapeutic properties in traditional medicine across India and beyond. Originating from southern Europe, it is now cultivated commercially in various regions, including North America and Eastern Europe. Calendula flowers, with their vibrant colors ranging from cream to orange, possess unique properties beneficial for skin health, including cell rejuvenation, wound healing, and inflammation reduction. Its annual growth spans 9 to 20 inches, with bright green leaves and flowers measuring 2 to 3 inches in diameter. Cultivation is straightforward, with seeds planted outdoors in early stages, and the plant thrives in cooler growing seasons. Despite its historical use in Ayurvedic and Homeopathic medicine systems for treating various ailments, scientific validation of its efficacy remains ongoing. Calendula's diverse biological activities, including anti-inflammatory and antioxidant properties, make it a valuable ingredient in skincare products. Its botanical classification within the Asteraceae family[4,14].

2. Methodology

2.1 Extraction and Characterization of Calendula Officinalis L. Essential Oil

A. Extraction Process: The essential oil of Calendula officinalis L. was extracted using the steam distillation method. Calendula flower petals were cleaned, packed into a distillation flask with water and porcelain chips, and distilled for 8 hours. The collected oil underwent purification to remove residual water traces[5,6].

B. Physicochemical Evaluation: Physicochemical parameters of the oil were assessed, including specific gravity, viscosity, ester value, acid number, and saponification value, following Indian Pharmacopoeia protocols[7,21].

C. Characterization by GC-MS: Gas Chromatography-Mass Spectroscopy (GC-MS) analysis revealed various terpenoids and flavonoids in the oil, with 1,8 cineole and α-pinene identified as significant constituents[8].

D. Fingerprinting by HPLC: High-Performance Liquid Chromatography (HPLC) was employed to characterize the oil further. Notably, 1,8 cineole and α-pinene were quantified using an isocratic system, with optimized parameters detailed[12].

E. Formulation of O/W Sunscreen Cream: Five O/W emulsions containing different concentrations of Calendula oil were formulated using selected cosmetic bases and ingredients, with meticulous attention to stability and quality[15,20].

F. Physical Stability Parameters Determination: Various stability tests, including centrifugation, globule size analysis, zeta potential, conductivity, pH, and viscosity measurements, were conducted to ensure the stability and integrity of the formulations under different conditions.

G. In vitro SPF Testing: Sun Protection Factor (SPF) values of the formulations were determined using the in vitro method, demonstrating significant sun protection activity, particularly in formulations with higher concentrations of Calendula oil.

This comprehensive study highlights the extraction, characterization, formulation, and evaluation of Calendula officinalis L. essential oil, emphasizing its potential applications in skincare formulations with sun protection properties[9,10,11,21].

Table 1: HPLC parameters for 1,8 cineole and α-pinene analysis

Particulars	Detail
Columns	*C18(2) RP,250X4.6MM (Phenomenex-Luna column*
Guard column	33x4.6mm (Phenomenex-Luna column
Mobile phase	Methanol: H2O: Tetrahydrofuran (THF)
(50:46:4) Flow rate	1ml/min
Wavelength	292nm
Injection volume	20µl
Retention time	6.81min for 1,8 cineole and 7.32 for α-
pinene Run time	10 min

Table 2: Concentration and area of standard 1,8 cineole and α-pinene

S. No.	Conc.(µg/ml)	Area	Conc.(µg/ml)	Area
1	10	19826.8	10	33490.8
2	20	42304.1	20	70489.6
3	30	57169.3	30	140118.4
4	40	78482.3	40	136592.4
5	50	99481.9	50	156056.4
6	60	104558.2	60	195493.8
7	70	124642.2	70	225420.6
8	80	144903.7	80	265687.5
9	90	159985.5	90	301728.7
10	100	186443.1	100	348119.4

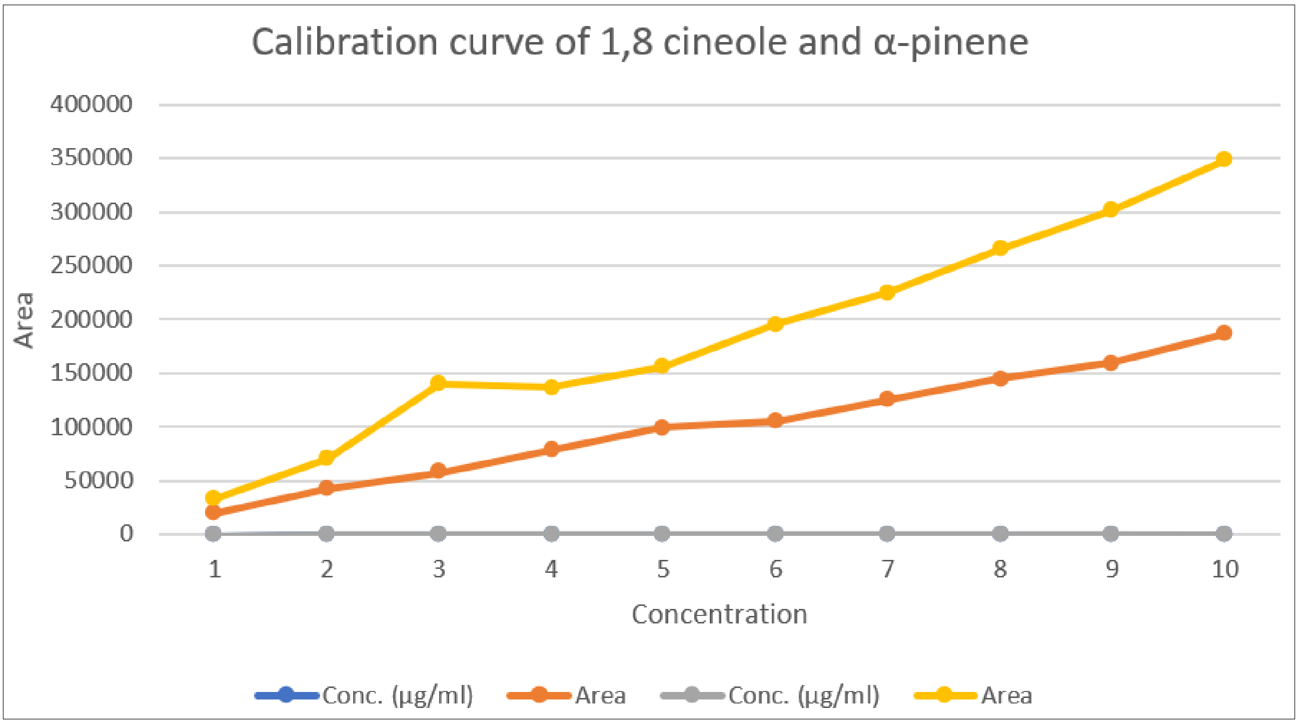

Figure 2: Calibration curve for standard 1,8 cineole and α-pinene

3. Result and Discussion

A. Extraction Yield and Physicochemical Parameters: The extraction yield of essential oil from Calendula officinalis L. was determined to be 1.25%. Following extraction, various physicochemical parameters including specific gravity, viscosity, ester value, acid number, and saponification value were evaluated. The specific gravity was found to be 0.795 g/ml at 25°C, with a viscosity of 15 cP. Additionally, the ester value, acid number, and saponification value were measured at 113.43, 3.37, and 116.8, respectively.

B. HPLC Fingerprinting: HPLC analysis successfully identified and quantified 1,8 cineole and α-pinene in *Calendula* oil. The retention times for these compounds were determined, and the method underwent validation for various parameters including linearity, limit of detection, limit of quantification, accuracy, and precision.

The analysis method underwent validation for various parameters including linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision (both interday and intraday), as detailed in Table 6. The selection of 1,8 cineole and α-pinene for study was based on their recognition as the primary constituents of Calendula essential oil.

1,8-cineole and α-pinene are recognized as key constituents of Calendula essential oil with documented antioxidant properties, making them ideal candidates for our study. HPLC analysis clearly distinguished peaks corresponding to 1,8-cineole and α-pinene, showcasing excellent peak symmetry. The calculated concentrations of 1,8-cineole and α-pinene in Calendula essential oil were 8.12% and 22.535% w/w, respectively. These findings suggest a potential mechanism for maintaining balanced levels of lipid peroxidation and antioxidant enzymes, particularly relevant in addressing UVB-induced oxidative damage in the skin of albino rats.

Furthermore, our proposed analytical method was extended to evaluate the recovery of extracted components. Following the quantitative analysis and method validation, a recovery test was conducted post-formulation of a Calendula essential oil-based cream. This test aimed to assess any possible interactions between the formulation's components and Calendula essential oil, ensuring the cream's efficacy and stability.

C. Formulation of O/W Sunscreen Cream: A sunscreen cream was formulated using Calendula oil. The cream's formulation included various ingredients such as stearyl alcohol, beeswax, sorbitan monooleate, and Calendula oil, among others. Different concentrations of Calendula oil were tested to evaluate their effects on the cream's stability and efficacy.

Table 3: The formula adopted for topical formulation

Ingredients	**Concentration (w/v)**
Oleagenous Phase	
Stearyl alcohol	15%
Beeswax	8%
Sorbitan monooleate	1.25%
Calendula oil	1-5%*
Aqueous Phase	
Sorbitol solution 70% USP	7.5%
Polysorbate 80	3.75%
Methyl paraben	0.025%
Propyl paraben	0.015%
Deionized water, q.s.	100%

*For the formulation F1, F2, F3, F4 and F5, the concentration of Calendula oil was 1%, 2%, 3%, 4% and 5%.

Physical Stability Parameters: The physical stability of the formulated creams was assessed through stability tests, centrifugation tests, and globule size analysis. The creams demonstrated stability under various storage conditions, with no evidence of phase separation or significant alterations in color. Globule size analysis revealed consistent sizes and polydispersity indices, indicating homogeneity within the formulations.

D. Viscosity Measurement: Viscosity measurements were conducted to evaluate the consistency of the creams over time. The viscosity of the creams remained stable across different formulations and storage durations, indicating robust physical stability. During the viscosity assessment across all formulations, it was observed that an escalation in the concentration of Calendula oil, the active sunscreen agent, led to a corresponding increase in viscosity. However, upon evaluating the formulations stored at room temperature on the 3rd, 7th, and 15th days, the discrepancies in viscosity values were negligible. The highest viscosity was recorded in formulation F5 (83128 cP) after 15 days, while the standard formulation reached its peak viscosity (75854 cP) after the 7th day. The consistent viscosity values observed even after 15 days of measurement indicate robust physical stability across all cream formulations.

Table 4: The formula adopted for topical formulation

Formulation	Viscosity* (cP) at room temperature			
	Day 1	Day3	Day7	Day15
F_1	41586±25.45	41758±35.23	41219±32.15	41394±42.21
F_2	52186±45.25	52752±12.34	52479±28.25	52024±32.52
F_3	62859±34.12	62547±32.45	62895±36.55	62880±19.33
F_4	69521±45.10	70267±44.12	70349±69.21	72547±29.54
F_5	82016±52.16	82568±16.42	83128±35.21	82449±25.21
F_s	75698±12.26	75854±44.62	75485±19.52	75611±44.52
F_b (blank)	30254±16.32	31257±48.25	31859±14.25	31446±64.25

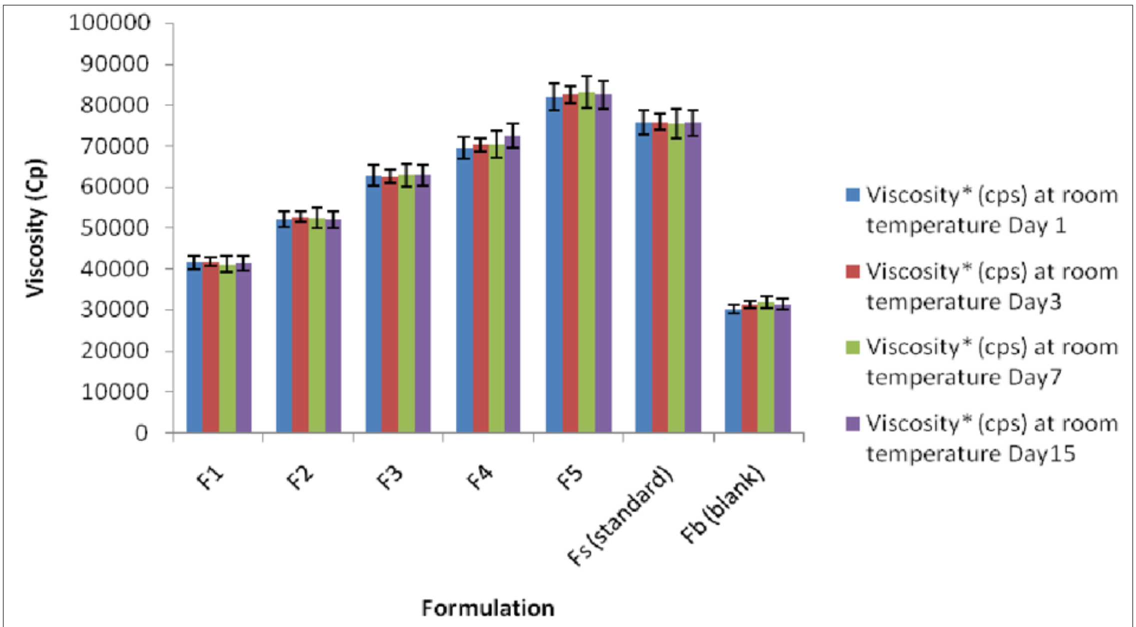

Figure 3: Graphical representation of effect of storage days on viscosity

Table 5: Result of SPF values by the in vitro method

Formulation	SPF Values (Mean±SEM)
F_1	4.51±0.17
F_2	6. 83±0.13
F_3	13.15±0.21
F_4	14.45±0.10
F_5	14.87±0.14
F_S	15.36±0.16

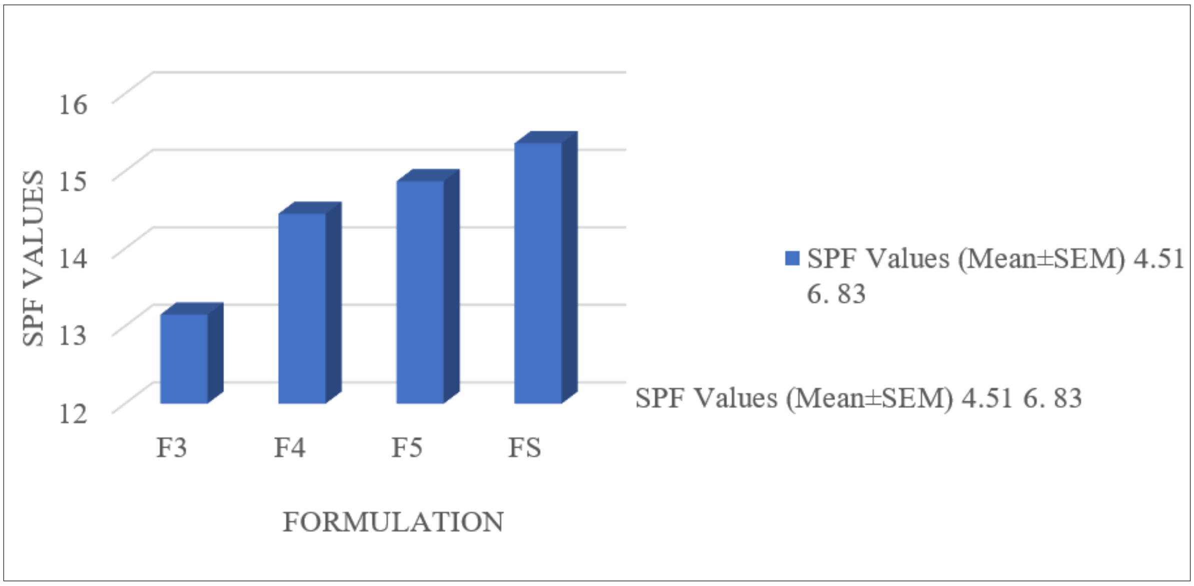

Figure 4: In vitro and in vivo SPF measurement of Formulations

*The data represent the mean ± SEM of three independent experiments. *p0.001 when compared with negative control (Fb),†p0.01 when compared with control (FS)*

E. SPF Values: The sun protection factor (SPF) values of the formulated creams were determined using in vitro methods. Formulations containing higher concentrations of Calendula oil exhibited higher SPF values, with formulation F5 demonstrating the highest SPF value of 14.87 ± 0.14. These findings suggest the potential of Calendula oil-based creams in providing sun protection.

No evidence was found in the literature regarding sun protection factor studies on formulations containing Calendula officinalis flower essential oil. However, it's noted that tribal communities traditionally use Calendula extract and oil for various dermatological conditions such as skin injuries and burns. The present study revealed a novel finding that formulations containing Calendula essential oil, particularly F3, F4, and F5, exhibit significant sun protection activity, potentially shielding the skin from harmful UVB radiation.

Both in vitro methods employed in this investigation yielded similar results, indicating a good correlation between them in measuring SPF. The SPF values of F3, F4, and F5 emulsions demonstrated their photoprotective potential, while F1 (4.51±0.17) and F2 (6.83±0.13) exhibited insignificant SPF values compared to F3, F4, and F5 and were therefore excluded from further study.

Previous studies have highlighted the excellent skin-penetrating properties of stable Calendula-based creams, suggesting their potential in wound healing and preventing protein loss from skin cells. In contrast, the control formulation (FS) based on sunflower oil, despite its triglyceride content, does not penetrate deeply into the skin due to its bulky structure, resulting in minimal impact on protein loss from the dermal surface.

Recent USFDA guidelines suggest that sunscreens with an SPF of 15 are sufficient when applied adequately to protect the skin from UVB radiation and reduce premature skin aging. Hence, formulations such as F3, F4.

4. Conclusion

The physicochemical evaluation of Calendula essential oil indicated favorable results within standard limits, establishing key parameters for its characterization. GC-MS analysis identified twenty-two compounds, predominantly terpenoids and flavonoids, with 1,8 cineole and α-pinene as significant components, known for their antioxidant properties. These findings suggest potential UVB protection in formulations. Stable oil-in-water creams incorporating 1-5% Calendula oil showed no phase separation, with consistent viscosity and globule size indicating stability. Zeta potential values suggested high stability and uniform particle distribution. No changes in viscosity after 15 days confirmed inherent stability, with no interaction between components. The study highlights the cost-effectiveness of Calendula oil extraction and its notable sun protection activity in creams, confirmed by in vitro SPF results. Formulations with 1% and 2% oil were excluded due to low SPF, while F5 showed comparable SPF to the control without synthetic sunscreen agents. These findings endorse Calendula-based formulations as promising natural alternatives for sun protection in cosmetics, offering a sustainable approach without synthetic additives.

Conflict of Interest: None

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