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Original Article

# Formulation and Characterization of a Polyherbal Sunscreen Containing *Camellia sinensis*, *Vitis vinifera*, and *Silybum marianum* Extracts

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## ABSTRACT

**Background and Objectives:** Prolonged Ultraviolet (UV) exposure induces oxidative stress, leading to inflammatory skin disorders in photosensitive individuals. This study aimed to develop and evaluate a polyherbal sunscreen cream containing *Camellia sinensis* (CS), *Vitis vinifera* (VV), and *Silybum marianum* (SM) for its antioxidant and photoprotective efficacy. **Materials and Methods:** Alcoholic extracts of CS, VV, and SM were incorporated into six topical Formulations (F1-F6) using stearic acid as an emulsifier. Formulations were assessed for pH, spreadability, viscosity, drug content, total phenolic content, *in vitro* drug release, antioxidant activity, and Sun Protection Factor (SPF). FTIR analysis confirmed compatibility, while HPTLC validated the presence of key phytochemicals-epicatechin-3-galactoside (CS), procyanidin (VV), and Silybin (SM). **Results and Discussion:** Among the formulations, F4 demonstrated superior performance with 82.97±3.52% w/w of CS, 86.44±3.74% w/w of VV, and 80.78±31.76% w/w of SM. *In vitro* release studies indicated 75.77±3.59% w/w of CS, 78.12±4.23% w/w of VV, and 74.71±3.95% w/w of SM release over 8 hr, following zero-order kinetics and Higuchi diffusion. F4 exhibited a total polyphenolic content of 30 mg (Eq.GA)/g and 71.56±2.89% ROS inhibition, signifying potent antioxidant activity. Its SPF of 25.384 matched synthetic sunscreens. Stability studies confirmed its robustness over three months. **Conclusion:** F4 demonstrated excellent antioxidant and photoprotective properties, offering a promising natural alternative for managing UV-induced skin damage in photosensitive individuals.

**Keywords:** Polyherbal sunscreen, Photosensitive skin disorders, Antioxidant, Sun Protection Factor (SPF), and *in vitro* drug release.

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## INTRODUCTION

Photosensitive skin disorders are a significant concern in dermatology, affecting a substantial portion of the population, particularly in regions characterized by intense solar radiation.[1] These disorders manifest as aberrant cutaneous reactions to Ultraviolet (UV) radiation, resulting in erythema, pruritus, and inflammation in sun-exposed areas.[2] Such conditions underscore the necessity for efficacious sunscreens that not only provide adequate physical protection against deleterious UV rays but also possess inherent antioxidant properties to mitigate oxidative stress and attenuate UV-induced skin damage.[3] The prevalence of photosensitivity has accentuated the critical demand for innovative formulations that can enhance cutaneous resilience and provide sustained photoprotection.[4] Ultraviolet radiation is a primary environmental factor contributing to various dermatological disorders, with distinct effects attributed to three classifications: UVA, UVB, and UVC. UVA rays penetrate deeply into the dermis and are implicated in photoaging and the development of cutaneous malignancies.[5] Conversely, UVB rays primarily affect the superficial epidermal layers, inducing erythema, and increasing the risk of neoplasms.[6] The adverse effects of UV

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exposure are largely mediated by the generation of Reactive Oxygen Species (ROS), which can cause immediate cutaneous damage and promote long-term structural and functional alterations. Although conventional sunscreen formulations aim to mitigate these risks, they often fail to provide adequate protection without causing skin irritation and allergic reactions due to their synthetic chemical ingredients.[7] This necessitates the exploration of alternative strategies to improve the efficacy and safety of sun-protection products.

Recent studies have elucidated the potential of traditional Chinese medicinal plants as effective photoprotective agents due to their inherent antioxidant properties.[8] Notably, *Camellia sinensis* (Chinese tea),[9] *Vitis vinifera* (grape),[10] and *Silybum marianum* (milk thistle)[11] have rich phytochemical profiles, particularly polyphenols, which effectively neutralize free radicals and reduce oxidative damage to skin cells. *Camellia sinensis*, widely used in traditional Chinese medicine, is renowned for its high catechin content, which offers protection against UV-induced skin damage.[12,13] *Vitis vinifera*, while not native to China, is frequently integrated into traditional Chinese medicinal practices and contains resveratrol and proanthocyanidins that contribute to dermal health and possess notable photoprotective effects.[14,15] Additionally, *Silybum marianum* is recognized for its potent antioxidant activity, potentially enhancing the skin's endogenous defence against UV radiation.[16,17]

Despite the known benefits of these herbal extracts, their specific combination in sunscreen formulations is underexplored, indicating a significant gap in the current literature. This study aimed to address this gap by formulating a polyherbal sunscreen that leverages the synergistic effects of these traditional Chinese medicinal plants, evaluating its antioxidant mechanisms, and determining its Sun Protection Factor (SPF) efficacy. These herbal extracts were selected based on their unique properties and traditional applications in Chinese herbal medicine. This innovative polyherbal combination seeks not only to enhance photoprotection through their collective antioxidant activities but also to fulfil the demand for safer and more effective sunscreen options. The theoretical framework of this study is founded on the understanding that antioxidants mitigate UV-induced damage at the cellular level by neutralizing reactive oxygen species generated during UV exposure, thereby safeguarding cellular components from oxidative stress and preventing skin damage.[18,19] This protective mechanism underscores the significance of selected traditional Chinese medicinal plants in developing an effective sunscreen.

The objectives of this research were threefold: (1) to elucidate the antioxidant mechanisms of the bioactive compounds found in the selected herbal extracts and their synergistic effects; (2) to formulate a novel polyherbal sunscreen that incorporates these extracts and evaluate its physicochemical properties; and (3) to assess the SPF efficacy of the formulated sunscreen and determine its potential as a protective agent for individuals with photosensitive skin disorders. By adopting a systematic approach to examine these objectives, this study aimed to provide valuable insights into the efficacy and safety of herbal-based sunscreens, thereby contributing to the ongoing exploration of natural alternatives in dermatological care. The methodology employed in this research is innovative, as it seeks to incorporate advanced techniques for evaluating both antioxidant mechanisms and SPF efficacy, potentially enhancing the existing methods used in sunscreen research. Ultimately, the findings from this research could pave the way for the development of effective, natural sunscreen products that cater specifically to individuals with photosensitive skin disorders. By elucidating the antioxidant mechanisms and SPF efficacy of a polyherbal sunscreen formulated with *Camellia sinensis, Vitis vinifera, and Silybum marianum*, this study aimed to advance our understanding of herbal sunscreens and offer new avenues for protecting against photosensitive skin disorders, thereby improving the health and quality of life of affected individuals.

## MATERIALS AND METHODS

### Plant Extract Materials and Phytochemical Screening

The plant extract of *Camelia sinensis* (CS) was obtained from Synthite Industries Private Limited, Synthite Valley, Kolenchery, Kerala. *Vitis vinifera* (VV) and *Silybum marianum* (SM) were procured from Sreedha Phyto Extracts, Jaipur, and Rajasthan. Phytochemical screening was performed to identify key bioactive compounds in the extracts. Qualitative tests were conducted following the standard protocols for the detection of various phytochemicals. The presence of alkaloids, flavonoids, carbohydrates, tannins, phenolic compounds, glycosides, and saponins was also assessed.[20,21]

### Preparation of Standard Curve

The UV range between 200 and 800 nm was scanned for the absorption maxima of ethanolic extracts of CS, VV, and SM individually using a Shimadzu UV-1700 series spectrophotometer. Each extract (100 mg) was accurately weighed and transferred to a 100 mL volumetric flask. The volume was adjusted to 100 mL by using ethanol. A standard solution was prepared by transferring 10 mL of this solution to a 50 mL volumetric flask. Aliquots of 1, 2, 3, 4, and 5 mL of this solution were transferred to five 10 mL volumetric flasks to obtain sample solutions with concentrations of 10, 15, 20, 25, and 30 µg/mL, respectively. The absorbance of each solution was measured at its respective absorption maxima. Subsequently, a calibration curve of the concentration versus absorbance was constructed for CS, VV, and SM.[22,23]

### Compatibility Studies using FTIR Spectrophotometer

Fourier Transform Infrared spectral analysis was employed to investigate the compatibility of herbal extracts of CS, VV, and SM

with stearic acid and cetyl alcohol. Spectral analysis of the herbal extracts, stearic acid, cetyl alcohol, and a combination of herbal extracts with stearic acid and cetyl alcohol, was conducted to examine potential alterations in the chemical composition of the raw materials following their combination with these excipients.[24-26]

## HPTLC analysis of Plant Extracts

To prepare the standard and sample solutions, Epicatechin-3-galactoside was solubilized in 10 mL water to create a standard solution of oleic acid (100 mg/mL). The CS extract was accurately weighed and added to a 10 mL volumetric flask, and the volume was adjusted with water. HPTLC plates with 0.2 mm thickness silica gel 60 F254 and dimensions of 20 × 10 cm were utilized for the experiment without any prewashing. Using a Camag Linomat V automatic sample applicator, the samples were transferred to the plates as 8 mm bands that were spaced 8 mm apart and 10 mm from the plate's edges. In a Camag twin-trough glass chamber with a stainless-steel top, the plates were developed using the ascending technique to a thickness of 80 mm at a temperature of 25±5°C and relative humidity of 50 - 60% while employing a mobile phase composed of toluene: ethyl acetate (9:1) as the solvent. The chamber saturation time was maintained at 20 min. After development, a hot-air dryer was used to dry the plates. The plates were subsequently examined in a Camag UV cabinet and analyzed with a Camag TLC Scanner using the Win CATS program in the absorbance mode with slit dimensions of 6.00x0.45 mm. A detection wavelength of 550 nm was used.[27] Similarly, HPTLC analyses of the VV and SM were performed. For VV, procyanidin B2 was used as a marker compound to prepare the standard solution, and visualization was conducted at 254 nm. The solvent system was Toluene: Ethyl acetate (3:7). For SM, silybin was used as a marker compound for the preparation of the standard solution and visualization was conducted at 254 nm. The solvent system used was a Toluene: Ethyl acetate (1:9) mixture.[28]

## Formulation of a Polyherbal Cream

To prepare the polyherbal cream, ethanolic extracts of *Camelia sinensis* (CS), *Vitis vinifera* (VV), and *Silybum marianum* (SM) were incorporated into castor oil. Sunscreen cream was prepared using the following procedure: The initial step involved the preparation of the aqueous phase, where methylparaben, glycerine, and triethanolamine, three water-soluble components, were dissolved in distilled water and heated to 75°C. In the second step, the oil phase was prepared by adding medicinal oil to a mixture of stearic acid and cetyl alcohol and heating it to 75°C. The oil phase was subsequently introduced into the water phase at 75°C with continuous stirring for 15 to 20 min. The emulsion was then homogenized to achieve a uniform dispersion. The final product, which exhibited a cream-like consistency, was stored at 37°C in a wide-mouthed container. The Formulations (F1-F6) were prepared by varying the concentrations of the herbal extracts and emulsifying agents to assess their effects on the characteristics and efficacy of the cream.[29,30] A summary of the formulation composition is presented in Table 1.

## Evaluation of a Polyherbal Cream

### *Organoleptic Properties*

The colour, homogeneity, grittiness, and appearance of the prepared formulations were visually examined.[31]

## Determination of pH

Approximately 0.5 g of cream was weighed and diluted in 50 mL of distilled water to determine the pH using a digital pH meter. To obtain an accurate reading, the electrode must be thoroughly cleaned and free of any acid or alkali residues. The pH meter was calibrated using a standard buffer solution.[32]

## Viscosity Determination

A Brookfield Viscometer DV-II was used to measure the viscosity of the prepared formulation. Using spindle number 64, the formulation was poured into the adaptor of the viscometer, and the optimal working conditions were established. Viscosity was then directly measured at a speed of 6 rpm while maintaining a constant torque.[33] The following equation was used to determine the viscosity in centipoises:

$$\text{Viscosity} = \text{Dial Reading} \times \text{Factor for DV II at 6 RPM Factor was 1M (1000)}$$

## Spreadability Determination

To assess spreadability, an excess of the formulation was placed between two glass slides, and a 100 g weight was applied to the upper glass slide for 10 min to compress the formulation uniformly to a specific thickness. Subsequently, the edges were scraped to remove the excess cream. To measure spreadability, a 100 g weight was placed on the pan, and the time required for the separation of the two slides was recorded in seconds.[34] The following equation was used to determine the spreadability,

$$S = M * L / T$$

where L-Length of a glass slide, M-Weight tied to the upper slide, and T-Time.

## Determination of Drug Content

Five grams of cream were dissolved in 20 mL of ethanol. The solution was transferred to a 100 mL volumetric flask and diluted with ethanol to a specified volume. The solution was filtered through Whatman filter paper (No. 41) subsequently, 1 mL of the filtrate was transferred to a 25 mL volumetric flask and diluted to capacity with distilled water. Finally, the absorbance was measured using a UV Spectrophotometer.[35]

### *In vitro* Drug Release Study

A Franz diffusion technique was employed to evaluate the *in vitro* drug release of the bioactive compounds from the polyherbal formulations. Phosphate buffer (pH 7.4) was used as receptor medium. The diffusion cell assembly was covered with a synthetic cellulose membrane that was pre-soaked in receptor media overnight. The donor compartment contained 1 g of the formulation, which was maintained on a Franz diffusion apparatus at 37°C, with agitation at 700 rpm. At predetermined time intervals (0, 1, 2, 3, 4, 5, 6, and 8 h5), aliquots (0.5 mL) were withdrawn and immediately replaced with an equal volume of fresh buffer solution. Aliquots were diluted appropriately with the dissolving liquid before UV spectrophotometric analysis. To elucidate the mechanism of herbal drug release from the polyherbal cream, the data from the *vitro* diffusion studies were fitted to various kinetic equations, including zero order, first order, Higuchi, and Korsmeyer-Peppas equations.[36-38]

### Total Polyphenolic Content

The Gallic Acid Equivalence (GAE) method, which utilizes a combination of phosphomolybdate and phosphotungstate, was employed for the colorimetric assessment of phenolic and polyphenolic antioxidants. This method quantifies the amount of substance required to inhibit reagent oxidation. Standard gallic acid (0.10-5 mg/mL in water) was prepared to construct the calibration curve, and 1 mg/mL of the extract solution was added. Folin-Ciocalteu reagent and 7% sodium carbonate solution were added to 1 mL of each sample. The mixture was allowed to react for 40 min at room temperature. Following the reaction period, the components were combined and the resultant blue colour was measured spectrophotometrically at 725 nm. Total phenol content was calculated as gallic acid equivalents using a calibration curve.[39]

$$T=C^{*}V/M$$

Where, T-Total content of phenolic compounds (mg/g of plant extract); C-Gallic acid concentration (mg/mL); V-Extract volume (mL); M-Amount of plant extracts (g).

### Antioxidant Activity

The sample extract reduced the absorbance at 517 nm of a coloured DPPH solution in methanol, which was used to evaluate antioxidant activity. 75 µL of DPPH standard solution (1.3 mg/mL in methanol) was added to 3 mL of methanol and its absorbance was recorded. After 30 min, a decrease in absorbance was observed due to the herbal sample extract and the standard at various concentrations. Methanol was used as the blank instead of the sample extract. After 30 min, a UV-visible spectrophotometer (Systronic double beam-UV-2201) was used to measure absorbance at 517 nm.[40-42] A lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The equation used to determine the ability to scavenge DPPH radicals was:

$$\% \text{ inhibition}=C\text{-}T/C^{*}100$$

Where C-Absorbance of DPPH alone; T-Absorbance of DPPH along with various concentration levels of extract.

### UV Spectrophotometric Estimation of Sun Protection Factor

One gram of the prepared cream was transferred to a 100 mL volumetric flask and diluted with ethanol to the appropriate volume. Using ethanol as a blank, the absorption spectra of the formulated components were measured in the 290-450 nm regions. The absorbance values were recorded at intervals of 5 nm between 290 nm and 320 nm. The formula used to determine the SPF of the developed cream was SPF=CF290-320 EE (λ)×I (λ)×Abs (λ), where C is the correction factor, E is the erythmogenic effect of light with wavelength, I is the solar intensity spectrum, and Abs is the spectrometric absorbance values at 290-320 nm. The EE*I value was constant. Aliquots were scanned between 290 and 320 nm. The absorbance values were obtained by multiplying the resultant EE (λ) by I(λ). These values were summed, and the results were multiplied by the adjustment factor.[43,44]

### Stability Studies

Accelerated stability studies were conducted to assess the stability of the polyherbal cream. The samples were stored for three months in a stability chamber (Remi, India) at 25°C with 60% RH and at 40°C and 75% RH by ICH recommendations. Samples were collected at 0, 1, 2, and 3 months. The percentage of drug content, as well as any alterations in their physical characteristics and chemical stability throughout storage, were subsequently evaluated.[45,46]

## RESULTS

### Phytochemical Screening

Phytochemical analysis confirmed the presence of key bioactive compounds in all extracts. Flavonoids and phenolic compounds were present in all three extracts. Alkaloids were detected in CS and VV but were absent in SM. Carbohydrates were found in VV and SM, whereas tannins were observed in all extracts, contributing to their potential antioxidant properties. Glycosides were identified only in VV, and saponins were detected in VV but were absent in CS and SM.

### Absorption Maxima

The UV-visible spectroscopic analysis revealed distinct absorption maxima for each extract: CS at 273 nm, VV at 327 nm, and SM at 284 nm (Figure 1). These absorption peaks corresponded to the presence of phenolic compounds, indicating their potential bioactivity. Standard calibration curves for CS, VV, and SM

were generated by plotting the absorption maxima (Figure 2). These standard plots served as a reference for quantifying the concentration of phenolic compounds in the formulations, allowing for a more precise evaluation of their bioactive potential.

## FTIR Analysis

### *FTIR Analysis of Cetyl Alcohol and Stearic Acid*

The FTIR spectra of cetyl alcohol and stearic acid exhibit characteristic peaks that provide insight into their molecular structures. For cetyl alcohol, a broad O-H stretch appears around 3324 $cm^{-1}$, indicating the presence of Hydroxyl (-OH) groups typical of fatty alcohols. Peaks in the range of 2958 $cm^{-1}$ represent C-H stretching vibrations from the long aliphatic chain, while bending vibrations of C-H bonds are observed around 1468 $cm^{-1}$. The spectrum also features a C-O stretch near 1060 $cm^{-1}$, confirming the alcohol functional group. In contrast, stearic acid displays a similar broad O-H stretch in the same range but also features a distinct carbonyl (C=O) stretch around 1710 $cm^{-1}$, indicative of the carboxylic acid functional group. Additionally, C-H stretching vibrations appear at approximately 2900 $cm^{-1}$ and bending vibrations around 1464 $cm^{-1}$, further confirming the long-chain aliphatic structure of stearic acid and reinforcing its fatty acid nature.

### FTIR Analysis of *Camellia sinensis*

The FTIR spectrum of *Camellia sinensis* shows a broad O-H stretch around 3275 $cm^{-1}$, indicative of phenolic compounds and flavonoids present in the extract. The presence of C=C stretching vibrations around 1609 $cm^{-1}$ suggests the existence of aromatic compounds, which is consistent with the flavonoids identified in the phytochemical analysis. A distinct peak near 1695 $cm^{-1}$ may represent carbonyl (C=O) stretches associated with other phenolic compounds. The peaks within 1145 $cm^{-1}$ indicate C-O stretches, further confirming the presence of carbohydrates and glycosides.

### FTIR Analysis of *Vitis vinifera*

*Vitis vinifera* exhibits similar broad O-H stretching in the range of 3270 $cm^{-1}$, correlating with its flavonoid and phenolic content. Additionally, this extract displays C=C stretching at approximately 1599 $cm^{-1}$, reinforcing the presence of aromatic structures. The spectrum features notable peaks around 2972 $cm^{-1}$, likely attributed to the hydroxyl groups found in resveratrol, a significant bioactive compound. The C=O stretch around 1690 $cm^{-1}$ is indicative of the presence of both phenolic compounds and glycosides. Furthermore, the presence of C-O stretching vibrations within the range of 1118 $cm^{-1}$ suggests the occurrence of carbohydrates, which aligns with the phytochemical results.

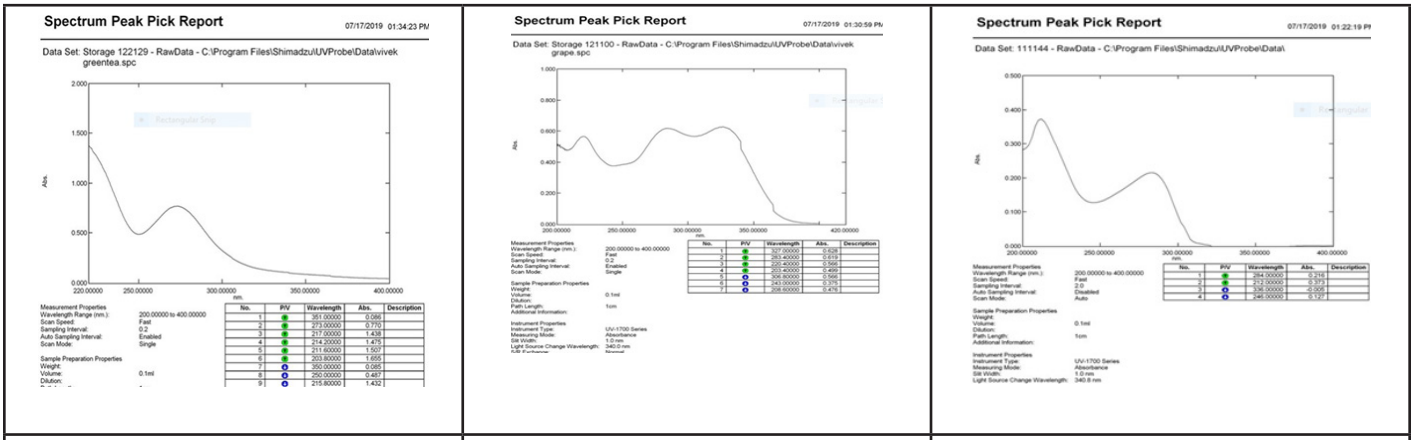


**Figure 1:** Determination of Absorption Maxima (λmax) of (a) CS, (b) VV, (c) SM.

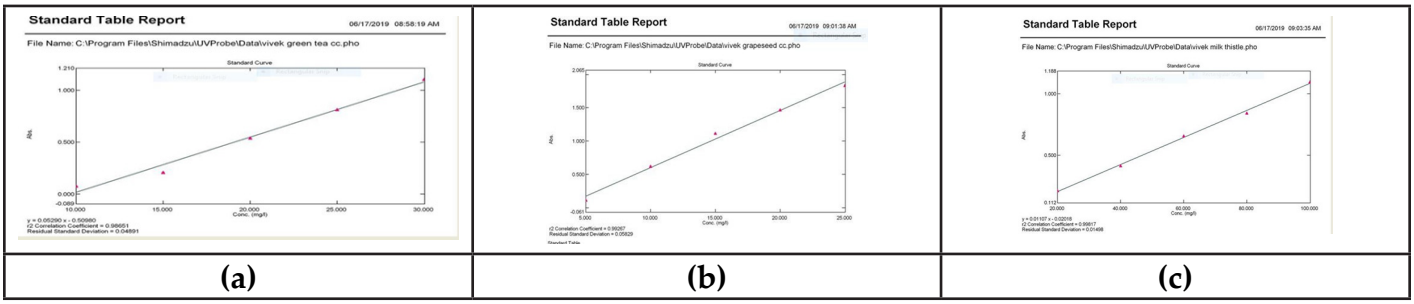


**Figure 2:** Construction of Standard Plot (a) CS, (b) VV, (c) SM.

## FTIR Analysis of *Silybum marianum*

The FTIR analysis of *Silybum marianum* reveals a broad O-H stretch around 3528 $cm^{-1}$, consistent with its phenolic compounds. The C=C stretching observed around 1615 $cm^{-1}$ supports the presence of flavonoids. A prominent peak near 1703 $cm^{-1}$ suggests the existence of carbonyl groups associated with silymarin, confirming the phytochemical finding of its presence. Additionally, C-O stretching within 1117 $cm^{-1}$ indicates the presence of glycosides, further corroborating the phytochemical analysis.

## FTIR Analysis of Herbal Extracts Combined with Fatty Acids and Alcohols

The FTIR analysis of the combinations of herbal extracts with fatty acids and alcohols demonstrated no significant interactions, indicating compatibility among the components (Figure 3). For (Figure 3A) Stearic Acid, *Camellia sinensis* (CS), and Stearic Acid+CS, the O-H stretch around 3275 $cm^{-1}$ (indicating the presence of phenolic compounds and flavonoids) and C=C stretching at 1609 $cm^{-1}$ (suggesting aromatic compounds) were preserved, alongside the carbonyl (C=O) stretch near 1710 $cm^{-1}$ from stearic acid (indicative of the carboxylic acid functional group). In (Figure 3B) Cetyl Alcohol, CS, and Cetyl Alcohol+CS, the broad O-H stretch at 3275 $cm^{-1}$ (indicating phenolic compounds) and C=C stretching at 1609 $cm^{-1}$ remained intact, confirming compatibility with cetyl alcohol, which exhibited its characteristic O-H stretch around 3324 $cm^{-1}$ (suggesting the presence of hydroxyl groups). For (Figure 3C) Stearic Acid, *Vitis vinifera* (VV), and Stearic Acid+VV, the O-H stretch at 3270 $cm^{-1}$ (correlating with flavonoids and phenolic compounds) and C=C stretching at 1599 $cm^{-1}$ (indicating aromatic structures) were retained, along with the C=O stretch around 1710 $cm^{-1}$ from stearic acid (indicative of the carboxylic acid functional group). In (Figure 3D) Cetyl Alcohol, VV, and Cetyl Alcohol+VV, the O-H stretch at 3270 $cm^{-1}$ (indicating flavonoids) and C=C stretching at 1599 $cm^{-1}$ remained unchanged, demonstrating compatibility with cetyl alcohol, which maintained its O-H stretch around 3324 $cm^{-1}$ (suggesting hydroxyl groups). For (Figure 3E) Stearic Acid, *Silybum marianum* (SM), and Stearic Acid+SM, the characteristic O-H stretch at 3528 $cm^{-1}$ (consistent with phenolic compounds) and C=C stretching at 1615 $cm^{-1}$ (supporting the presence of flavonoids) were preserved, along with the C=O stretch around 1710 $cm^{-1}$ of stearic acid (indicating carboxylic acid). Lastly, in

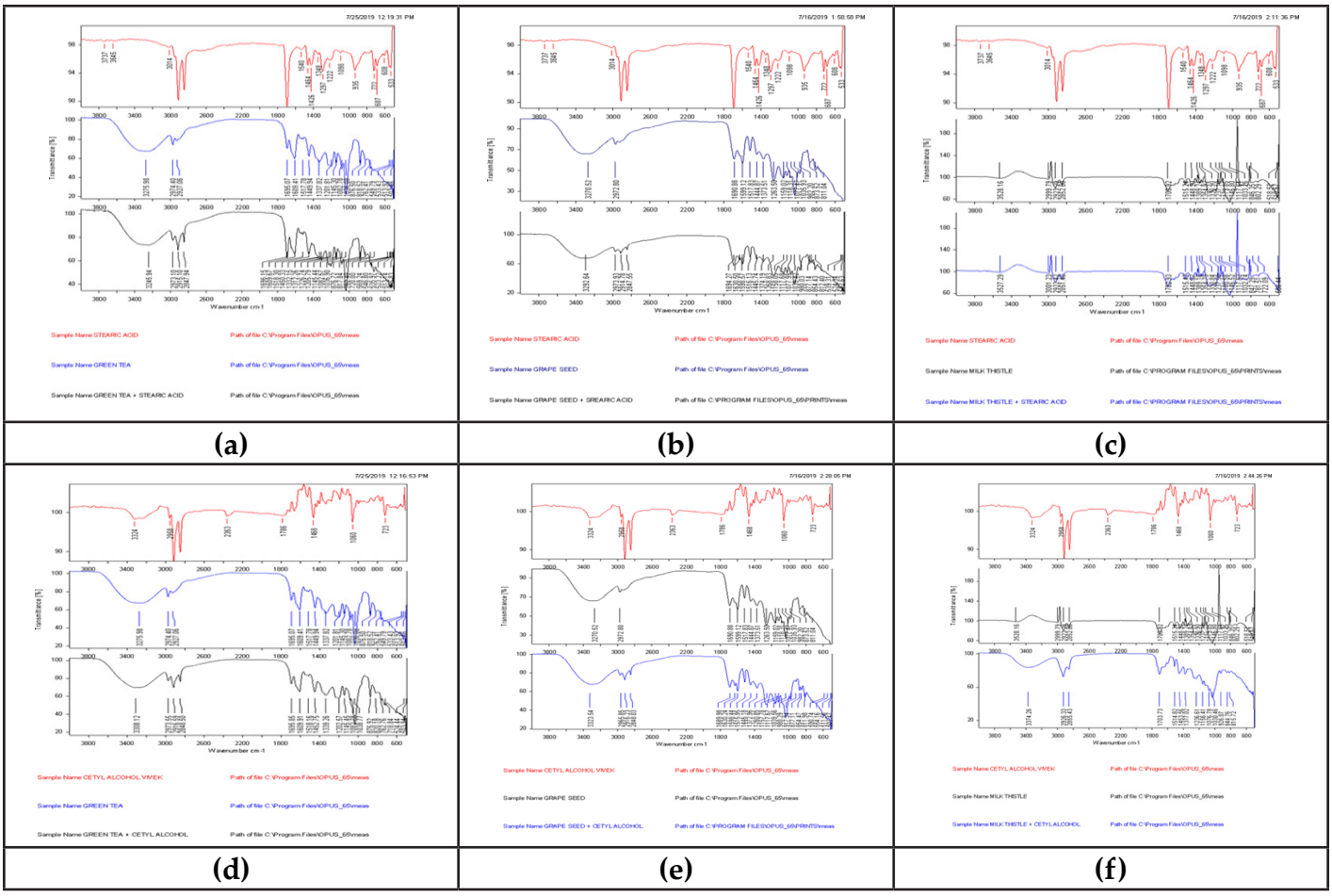


**Figure 3:** FTIR Spectrum of Drug-Excipient Mixture. CS+Stearic acid; (b) VV+Stearic acid; (c) SM+Stearic acid; (d) CS+Cetyl alcohol; (e) VV+Cetyl alcohol; (f) SM+Cetyl alcohol.

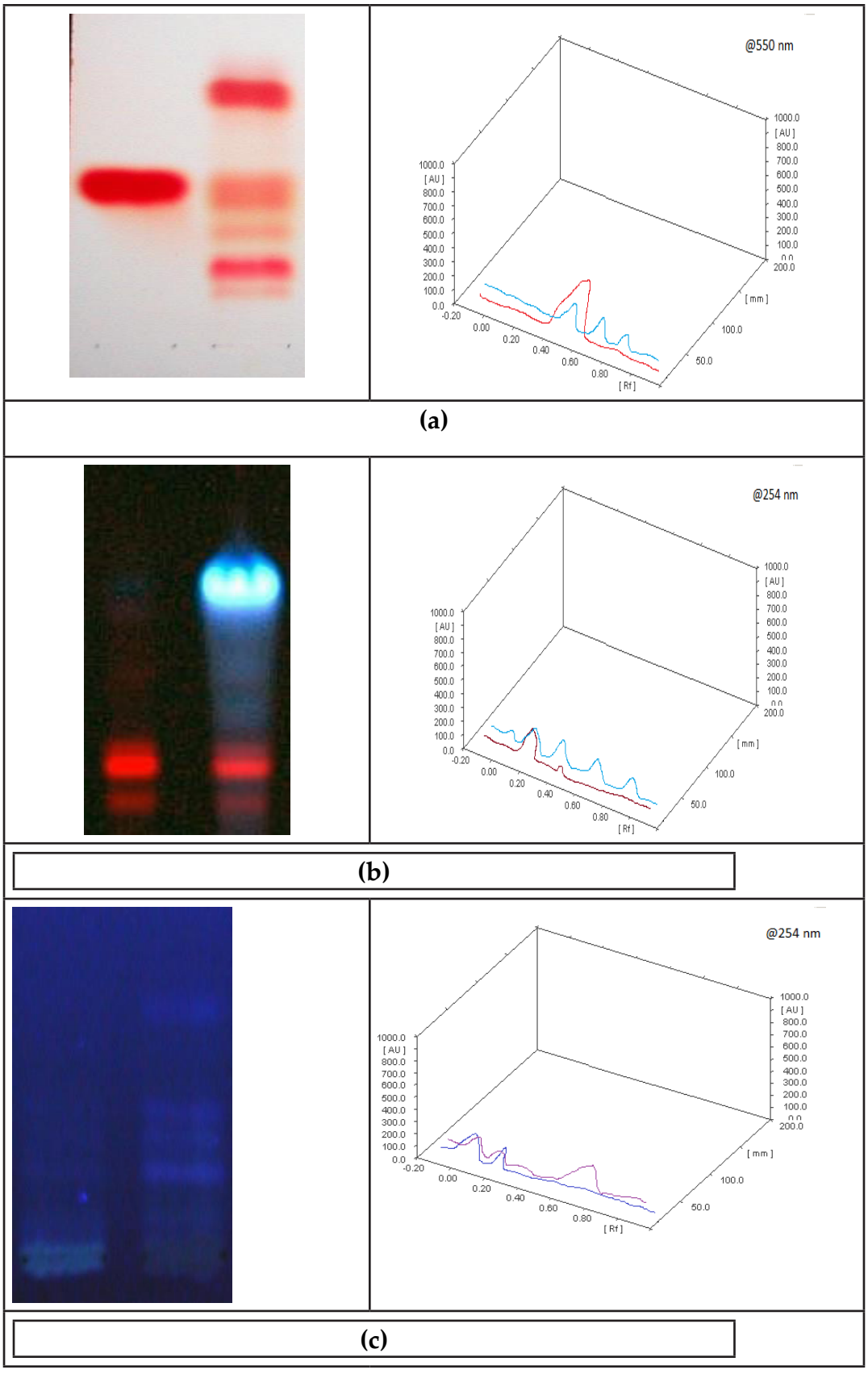


**Figure 4:** HPTLC and $R_f$ value of (a) CS, (b) VV, (c) SM.

(Figure 3F) Cetyl Alcohol, SM, and Cetyl Alcohol+SM, the O-H stretch around 3528 $cm^{-1}$ (indicating phenolic compounds) and C=C stretching at 1615 $cm^{-1}$ for SM were intact, indicating no interactions with cetyl alcohol, which retained its O-H stretch around 3324 $cm^{-1}$ (suggesting hydroxyl groups). Overall, the absence of significant peak shifts or new peaks in the FTIR spectra confirms the stability and compatibility of the herbal extracts with the fatty acids and alcohols used in the formulations.

## HPTLC analysis

The High-Performance Thin-Layer Chromatography (HPTLC) images presented in Figure 4 provide a comprehensive analysis of the key phytochemicals in the CS, VV, and SM extracts. In the CS extracts (Figure 4A), a distinct red band was observed at an $R_f$ value of 0.50, which closely aligned with the standard $R_f$ value (0.56 for epicatechin-3-galactoside, thereby confirming the presence of this important compound. VV extract (Figure 4B), the chromatographic analysis reveals a bright fluorescent blue band under UV light, corresponding to an $R_f$ value of 0.20, which is near the standard value of 0.25 for procyanidin, a marker compound for this extract. Similarly, SM extracts (Figure 4C) showed a clear peak with an $R_f$ value of 0.20, closely resembling the standard value of 0.28 for silybin, thereby confirming its presence in the extract. Collectively, these HPTLC results not only provide visual confirmation but also quantitative evidence of the active phytochemicals presents in the polyherbal formulation, enhancing our understanding of its pharmacological potential.

## Formulation Evaluation

Evaluation of the formulations showed that all samples possessed desirable physical characteristics. Formulation F4, with a creamy orange colour, had a pH of 5.81, ensuring skin compatibility and a viscosity of 30321±352.5 cps, which allowed for a smooth and uniform application. The spreadability of F4 was the highest among all formulations at 20.89±2.98 g.cm/sec, confirming its ease of application. Additionally, no phase separation or grittiness was observed in any formulation, demonstrating excellent homogeneity across all the samples. Formulation F4 was the most promising based on these evaluation parameters (Table 2).

**Table 1:** Formulation of Polyherbal Cream Containing Plant Extracts.

| Ingredients | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|
| CS (g) | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| VV (g) | 0.1 | 0.1 | 0.2 | 0.2 | 0.2 | 0.1 |
| SM (g) | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.2 |
| Cetyl alcohol (g) | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 | 1.2 |
| Stearic acid (g) | 0.4 | 0.8 | 1.2 | 1.6 | 2.0 | 2.4 |
| Glycerine (mL) | 5 | 5 | 5 | 5 | 5 | 5 |
| Methyl paraben (g) | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Castor oil (mL) | 2 | 2 | 2 | 2 | 2 | 2 |
| Triethanolamine | q.s. | q.s. | q.s. | q.s. | q.s. | q.s. |
| Water | q.s. | q.s. | q.s. | q.s. | q.s. | q.s. |

## Drug Content

Drug content analysis demonstrated that formulation F4 had the highest drug content among all formulations. Specifically, the content for *Camellia sinensis* (CS) was 82.97±3.52%, for *Vitis vinifera* (VV) it 86.44±3.74%, and for *Silybum marianum* (SM) it was 80.78±31.76%. These values reflect the efficient incorporation of the plant extracts into the cream, indicating F4's superior formulation compared to others, such as F1, with lower drug content percentages (70.87±1.23% for CS, 77.86±4.12% for VV, and 75.96±3.12% for SM).

## *In vitro* Drug Release

The *in vitro* drug release studies showed that F4 had the highest release rate (75.77±3.59%) for CS (Figure 5A), and 78.12±4.23% for VV (Figure 5B) and 74.71±3.95% for SM (Figure 5C). These results suggest that F4 is capable of sustained and effective release of the active compounds over time. F4 was chosen as the best formulation based on its superior drug content and other favourable properties. Further evaluations, including antioxidant activity, SPF determination, free radical scavenging, stability studies, and drug release kinetics, were conducted on F4 to comprehensively assess its potential as a promising formulation for therapeutic applications.

## Kinetics of Drug Release

The *in vitro* release kinetics of formulation F4, as depicted in Figure 5, demonstrated that drug release followed zero-order kinetics with an $r^2$ value of 0.951 for *Camellia sinensis* (CS), 0.990 for *Vitis vinifera* (VV), and 0.979 for *Silybum marianum* (SM). These high $r^2$ values suggested that the release rate remained constant over time, independent of the drug concentration. Additionally, the strong correlation with the Higuchi model ($r^2$ values of 0.951 for CS, 0.951 for VV, and 0.979 for SM) indicates that diffusion was the primary mechanism driving drug release, making it well-suited for sustained delivery. The data also showed a good fit with the Peppas model, with "n" values close to 1, further confirming the diffusion-controlled mechanism. These results support the potential of formulation F4 for controlled and sustained drug delivery.

## Polyphenolic Content

The absorbance value recorded at 1.012 further supported this finding, indicating the substantial presence of polyphenolic compounds in the formulation. The total polyphenolic content of 30 mg (Eq.GA)/g is directly associated with the enhanced antioxidant potential of the formulation, which contributes to its ability to neutralize free radicals and protect against oxidative

**Table 2: Evaluation of the Prepared Polyherbal Cream.**

| Formulation | Colour | Phase Separation | Grittiness | Homogeneity | pH | Viscosity (cps) | Spreadability (gcm/sec) |
|---|---|---|---|---|---|---|---|
| F1 | Yellowish Brown | None | None | Good | 6.06±0.53 | 25678±245.3 | 18.38±1.25 |
| F2 | Yellowish Brown | None | None | Good | 5.98±0.81 | 27767±221.6 | 18.20±0.85 |
| F3 | Creamy Orange | None | None | Good | 5.78±0.43 | 28987±315.3 | 20.23±2.01 |
| F4 | Creamy Orange | None | None | Good | 5.81±0.22 | 30321±352.5 | 20.89±2.98 |
| F5 | Light brown | None | None | Good | 6.04±0.13 | 33654±285.6 | 18.77±1.15 |
| F6 | Light brown | None | None | Good | 6.10±0.37 | 36553±272.9 | 19.58±2.41 |

**Table 3: Stability Studies for the Prepared Polyherbal Cream.**

| Temperature | Evaluation parameters | | Observation (months) | | | |
|---|---|---|---|---|---|---|
| | | | 0 | 1 | 2 | 3 |
| 40±2°C 75% RH | Physical appearance | | Creamy orange | No colour change | Slight colour change | Slight colour change |
| | Drug content (%w/w) | CS | 82.97 | 81.06 | 80.44 | 78.97 |
| | | VV | 86.44 | 85.62 | 83.77 | 80.59 |
| | | SM | 80.78 | 78.21 | 76.43 | 74.77 |
| | pH | | 5.81 | 5.75 | 5.69 | 5.64 |
| | Viscosity (cps) | | 30321 | 30118 | 30004 | 29912 |

damage. These results highlight the strong antioxidant properties of F4, which could be attributed to the presence of polyphenols.

## Free Radical Scavenging

The free radical scavenging ability of F4 was evaluated using the DPPH assay, which measures the percentage inhibition of DPPH radicals at various concentrations of the formulation. F4 demonstrated a dose-dependent increase in its antioxidant capacity, with higher concentrations showing greater inhibition of free radicals. At 100 µg/mL, F4 showed 49.97±2.13% inhibitions indicating moderate antioxidant activity. As the concentration increased, the scavenging activity significantly improved, with 56.25±1.75% inhibition at 200 µg/mL and 63.49±1.52% at 300 µg/mL. The highest concentration tested (400 µg/mL) resulted in a strong inhibition of 71.56±2.89%. These data highlight the superior antioxidant potential of F4, suggesting that it is highly effective in neutralizing free radicals, and could provide substantial protection against oxidative stress when used in formulations. These findings support the role of F4 as a potent free radical scavenger, potentially contributing to its overall protective and anti-ageing properties

## Sun Protection Factor (SPF)

*In vitro* SPF determination of F4 yielded promising results. The SPF value was calculated based on the absorbance of the UV radiation at different wavelengths (290-320 nm). The absorbance values were measured for each wavelength, and the corresponding erythemal effect (EE(λ)×I(λ)) was calculated. At 290 nm, the absorbance was 2.897, with an erythemal effect factor of 0.0150, resulting in an EE(λ)×I(λ)×Abs(λ) value of 0.0434. Similarly, at 305 nm, the absorbance was 2.245, contributing to a significant EE(λ)×I(λ)×Abs(λ) value of 0.735, indicating strong UV absorption in this range. The highest absorbance, recorded at 310 nm, was 3.056, corresponding to an EE(λ)×I(λ)×Abs(λ) value of 0.569. Overall, the data demonstrate F4's capacity to absorb UVB radiation effectively, which contributed to its SPF value of 25.384, comparable to that of synthetic sunscreens. These results underscore the potential of polyherbal creams as natural sunscreen formulations with significant photoprotective properties.

### Stability Studies

Stability studies for formulation F4, conducted over three months at 40ºC and 75% relative humidity, revealed that F4 remained relatively stable with no significant variations in key parameters (Table 3). The physical appearance of the formulation changed slightly, with an observable colour shift from creamy orange to a slightly altered colour after the second month, which persisted until the third month. Drug content analysis showed a gradual decrease for all three extracts: *Camellia sinensis* (CS) content decreased from 82.97% to 78.97%, *Vitis vinifera* (VV) from 86.44% to 80.59%, and *Silybum marianum* (SM) from 80.78% to 74.77% by the end of the third month. These reductions, although notable, remained within acceptable limits. The pH of the formulation showed a slight decrease, starting at 5.81 and reducing to 5.64 by the end of the study, indicating minimal acidification over time. The viscosity also exhibited a marginal decrease from 30,321 to 29,912 cps, maintaining an appropriate consistency throughout the study. These results indicated that despite minor changes in colour, drug content, pH, and viscosity, formulation F4 demonstrated good overall stability under accelerated storage conditions.

### Comparison of Formulation F4 with Commercial Sunscreens

In this study, the SPF value of F4 was determined to be 25.384, indicating that it is a potent natural photoprotective agent. For comparison, we evaluated two commercially available sunscreen creams: NIVEA Sun Protect and Moisture SPF 20, and Banana Boat Ultra Sport Sunscreen Lotion SPF 15. NIVEA Sun Protect and Moisture SPF 20 provides broad-spectrum protection against UVB rays while offering hydration owing to its Vitamin E content. However, its SPF value of 20 was lower than that of formulation F4, which indicates that F4 may offer superior protection against sunburn and long-term UVB damage. Similarly, Banana Boat Ultra Sport SPF 15 was formulated for active lifestyles, featuring a non-greasy texture and resistance to sweat and water. However, it's SPF of 15 limits its effectiveness in sun protection compared with F4.

A significant advantage of F4 lies in its incorporation of natural extracts, offering a safer alternative to chemical sunscreens, which are often associated with skin irritation, especially for sensitive skin types. The natural composition of F4 not only enhances its photoprotective properties but also reduces its environmental impact, addressing growing concerns about the harmful effects of chemical ingredients found in many commercial products. Moreover, if formulation F4 includes additional beneficial properties, such as moisturizing and antioxidant effects, it can serve as a multifunctional skincare solution that goes beyond sun protection. This aspect may particularly appeal to consumers seeking effective and safe options in the sunscreen market, reinforcing the potential of F4 as a competitive alternative to existing commercial sunscreens.

## DISCUSSION

This study successfully formulated a novel polyherbal cream incorporating the potent antioxidant properties of *Camellia sinensis* (CS), *Vitis vinifera* (VV), and *Silybum marianum* (SM) extracts for the treatment of photosensitive skin disorders. The formulation and evaluation process were aimed at ensuring the stability, efficacy, and safety of this herbal combination, with the goal of providing an effective alternative to conventional synthetic sunscreens.

Phytochemical analysis revealed the presence of bioactive compounds across all extracts, highlighting the richness of these herbal ingredients as antioxidants and other therapeutically active components. The consistent presence of flavonoids and phenolic compounds in the CS, VV, and SM extracts is significant, as these compounds are well-documented for their antioxidant and free radical-scavenging properties. The presence of alkaloids in CS and VV but their absence in SM is in line with the phytochemical diversity of these herbs. Moreover, the detection of carbohydrates in VV and SM and the presence of glycosides and saponins in VV further support the medicinal value of these plants. These findings indicate that each herb contributes distinct bioactive compounds, enhancing the overall therapeutic potential of the polyherbal formulation.

UV-visible spectroscopic analysis showed distinct absorption peaks for each extract (CS: 273 nm, VV: 327 nm, SM: 284 nm), which are consistent with the absorption characteristics of phenolic compounds. These absorption maxima confirmed the presence of phenolic and polyphenolic compounds, which were primarily responsible for the antioxidant activities observed in the formulation. The standard plots served as a reference for quantifying the concentration of phenolic compounds in the formulations, allowing for a more precise evaluation of their bioactive potential. The close correlation between the absorption peaks and phenolic content further reinforces the suitability of these extracts for sunscreen formulations, as phenolic compounds are known for their capacity to neutralize free radicals and absorb harmful UV radiation, making them suitable candidates for sunscreen formulations. The ability of the extracts to protect the skin from UV damage by absorbing UV rays may provide a natural alternative to synthetic UV filters.

FTIR analysis confirmed the chemical compatibility of the extracts with excipients used in the formulation. The absence of any significant chemical interactions or complexation between the active ingredients and excipients ensured the chemical stability of the final product. This stability is crucial for maintaining the efficacy of active compounds in the formulation, particularly

during storage and application. Ensuring the stability of extracts is crucial for preventing the degradation of bioactive compounds, which is essential for maintaining sustained antioxidant activity and effective photoprotection.

The HPTLC analysis further validated the presence of key phytochemicals in each extract. The presence of epicatechin-3-galactoside in CS, procyanidin in VV, and silybin in SM indicated that the formulation retained the essential bioactive components of the individual herbs. These compounds are known for their strong antioxidant properties, which play a significant role in neutralizing the Reactive Oxygen Species (ROS) generated by UV exposure. The accurate identification and quantification of these compounds using HPTLC also demonstrated the robustness of the extraction and formulation processes. $R_f$ values close to those of the standard compounds confirm that the bioactive components are well-preserved during formulation, ensuring their therapeutic efficacy.

Physical evaluation of the formulations revealed that all samples exhibited favourable characteristics for topical application. The pH values (ranging from 5.81-6.10) were within the acceptable range for skin application, ensuring that the formulation did not cause irritation or disrupt the natural pH balance of the skin. The viscosity values (25678±245.3-36553±272.9 cps) indicate a smooth and spreadable consistency, which is essential for ease of application. Additionally, the spreadability results suggested that the formulation could be evenly applied over the skin, enhancing its protective efficacy. The homogeneity observed in all formulations without any phase separation reflects the stability of the emulsions, which is crucial for the long-term performance of the cream.

Among the six formulations, F4 emerged as the most promising candidate, exhibiting the highest drug content in all three extracts (CS, 82.97±3.52%; VV, 86.44±3.74%; SM, 80.78±31.76%). This high drug content indicates efficient incorporation of active compounds into the formulation, which directly correlates with its therapeutic potential. The *in vitro* drug release study further supported the superiority of F4, with release rates of 75.77±3.59%, 78.12±4.23%, and 74.71±3.95% for CS, VV, and SM, respectively. Similar to the findings of Wang *et al*., where the *in vitro* release study of Nanostructured Lipid Carriers (NLCs) followed a sustained release profile and fit well with the Higuchi equation, our study also demonstrated a comparable sustained release pattern.[47] The release kinetics of our formulation aligned with the Higuchi model, indicating diffusion-controlled drug release from the matrix, which is ideal for ensuring prolonged therapeutic efficacy and consistent SPF protection over time.

The polyphenolic content analysis highlighted the superior antioxidant potential of formulation F4, which contained 30 mg (Eq.GA)/g, compared to 23.50% in F3. In comparison to Takayama *et al*.'s study, where the Rosmarinus officinalis hydroethanolic extract (ROe)-loaded emulgel exhibited a polyphenolic content of 24.15±0.11 mg Eq. GA/g, our formulation F4 demonstrated a higher polyphenolic content of 30 mg Eq. GA/g. This indicates that F4 possesses a greater concentration of polyphenols, which is directly linked to its enhanced antioxidant activity and UV protection. While Takayama *et al*. reported an SPF value of 7.56 for their ROE formulation; our F4 formulation achieved a much higher SPF of 25.384, further supporting its superior photoprotective efficacy.[48] The antioxidant activity observed in formulation F4 is 71.56±2.89%, which demonstrated by its superior free radical-scavenging ability, has significant implications for its photoprotective benefits. In comparison to Patki *et al*.'s study, where the orange pigment exhibited 52.36% DPPH inhibition and the yellow pigment showed 40.1% antioxidant activity, our formulation F4 demonstrated significantly higher antioxidant activity at 71.56±2.89%. Additionally, while Patki *et al*. reported relatively low SPF values of 5.3 and 2.60 for their pigments, our F4 formulation achieved a much higher SPF value of 25.384, indicating superior UV protection.[49] Free radicals generated by UV radiation are a major cause of skin damage, leading to premature ageing, DNA mutations, and an increased risk of skin cancer. Antioxidants play a crucial role in neutralizing free radicals, which are the major contributors to skin damage caused by UV exposure. By incorporating potent antioxidants, F4 provides a dual photoprotective mechanism: UV absorption through its SPF value, and protection against oxidative stress. This not only helps prevent immediate sun damage but also contributes to long-term skin health by preserving collagen and elastin, potentially reducing the visible signs of aging. The higher antioxidant capacity of F4 suggests that it offers better protection against oxidative stress, which is the primary cause of skin aging and UV-induced damage. The inclusion of polyphenolic compounds in the formulation provides dual benefits: direct protection by scavenging ROS, and indirect protection by stimulating the skin's natural defense mechanisms against UV radiation.

In comparison to Yen *et al*.'s study, which formulated a titanium dioxide-based sunscreen with SPF 20+ using emulsification, our F4 formulation achieved a higher SPF of 25.384, demonstrating superior UV protection. While their study relied on a metal oxide for UV reflection, our formulation incorporated natural extracts with both antioxidant activity and UV protection, providing a dual photoprotective mechanism.[50] The high SPF value indicates that F4 can effectively block UVB radiation, thereby reducing the risk of sunburn and long-term UV damage, including skin cancer. The use of natural extracts in F4 provides a safer alternative to chemical sunscreens, which are often associated with adverse effects, such as skin irritation and environmental harm.

Stability studies indicated that formulation F4 remained stable over three months of accelerated storage, with only minor changes in colour, pH, and viscosity. Similar results were reported by Cefali, *et al*.,[51] who studied the stability of emulsions containing Vitamin C in acerola extracts. During the stability tests, the pH values of the emulsions dropped to 4.02±0.3 due to the fact that Vit C is prone to thermal degradation at elevated temperatures and that light could decrease pH values during storage. Kim, *et al*.[52] also reported very low values of pH and a constant decrease (from 5 to 2) when studying the stability of L-ascorbic acid in O/W emulsions at 25ºC. Although there was a slight reduction in the drug content, it remained within acceptable limits, suggesting that the formulation maintained its efficacy over time. The slight decrease in pH and viscosity did not significantly affect the performance or safety of the formulation, indicating that F4 was a stable and reliable product for long-term use.

The successful formulation of F4, with its high antioxidant activity, SPF value, and stable characteristics, is a promising natural alternative for the treatment of photosensitive skin disorders. The polyherbal approach not only offers effective protection against UV radiation but also aligns with the growing demand for natural, eco-friendly skincare products. The integration of *Camellia sinensis*, *Vitis vinifera*, and *Silybum marianum* extracts into a single formulation leveraged the synergistic effects of their bioactive compounds, providing comprehensive skin protection. Moreover, the use of advanced analytical techniques, such as FTIR, HPTLC, and *in vitro* drug release studies, ensures the robustness and reliability of the formulation. By combining traditional herbal knowledge with modern pharmaceutical techniques, this study provides a precedent for future research on polyherbal formulations for dermatological applications.

## CONCLUSION

This study successfully formulated and characterized a novel polyherbal sunscreen that integrates the potent antioxidant properties of traditional Chinese herbs *Camellia sinensis, Vitis vinifera, and Silybum marianum,* addressing a significant gap in the current literature on herbal-based photoprotective agents. Preformulation studies, including FTIR analysis, demonstrated compatibility between the herbal constituents and excipients, whereas UV spectrophotometric studies established the optimal $\lambda_{max}$ of the extracts. The formulation process involved incorporating herbal extracts into the oil phase and optimizing the cream with emulsifying and buffering agents, resulting in six formulations, of which the F4 formulation exhibited superior characteristics. The synergistic effects of the combined extracts not only enhance the ability of the formulation to neutralize reactive oxygen species generated by UV radiation but also provide a multi-faceted approach to skin protection. This F4 formulation displayed excellent antioxidant activity, suggesting its potential efficacy as a photoprotective agent, which may mitigate the risk of skin cancer owing to its free radical-scavenging properties. By elucidating the mechanisms underlying the antioxidant activities of the selected herbs, this study provides valuable insights into their potential application in dermatological care. Assessment of the Sun Protection Factor (SPF) efficacy of the formulated sunscreen highlights its potential as a safe and effective alternative for individuals with photosensitive skin disorders. These results indicate that the herbal combination may offer superior protection against UV-induced damage compared to conventional formulations, promoting skin health and resilience. Furthermore, the innovative methodologies employed in this study establish a foundation for future studies exploring the mechanisms of action and efficacy of herbal sunscreens. This study reinforces the role of natural products in enhancing photoprotection and establishes precedents for the development of more effective, safer, and sustainable sunscreen formulations. These findings could significantly impact the formulation of dermatological products, fostering a shift toward herbal alternatives that align with the growing consumer demand for natural and environmentally friendly skincare options. By advancing our understanding of the protective benefits offered by these medicinal plants, this study opens new avenues for improving the quality of life of individuals affected by photosensitivity and other related skin disorders.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**CS:** *Camellia sinensis*; **VV:** *Vitis vinifera* (VV), **SM:** *Silybum marianum*; **SPF:** Sun Protection Factor; **UV:** Ultra Violet.

## SUMMARY

This study successfully formulated and evaluated a novel polyherbal sunscreen cream containing extracts of *Camellia sinensis*, *Vitis vinifera*, and *Silybum marianum* for photosensitive skin. Phytochemical screening confirmed the presence of key bioactive compounds in the extracts. Six Formulations (F1-F6) were prepared using stearic acid as an emulsifier. Formulation F4 demonstrated superior performance with high drug content, *in vitro* release, antioxidant activity, and SPF value. F4 exhibited favourable physical characteristics, polyphenolic content, and free radical scavenging ability. Stability studies confirmed its robustness over three months. The study highlights the potential of this polyherbal sunscreen as a promising natural alternative for managing UV-induced skin damage in photosensitive individuals.

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