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RESEARCH ARTICLE

Impact of Solvent Type on Total Flavonoid Content and Sun Protection Factors in *Centella asiatica* (L.) Urban Leaves Extract

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Abstract *Centella asiatica* (L.) Urb. is a widely recognized medicinal plant with neuroprotective, memory-enhancing, antioxidant, anti-inflammatory, anticancer, cardioprotective, and wound-healing properties. The leaves of *Centella asiatica* (L.) Urb. demonstrate antioxidant activity attributed to the presence of alkaloids, flavonoids, saponins, and tannins, making them promising candidates for natural sunscreen development. This study aimed to evaluate the total flavonoid content and sun protection factor (SPF) of the leaf extract *Centella asiatica* (L.) Urb. using UV-Vis spectrophotometry. The study was conducted through a series of experimental procedures, including raw material collection, simplicia preparation, extraction, fractionation, thin-layer chromatography (TLC), flavonoid quantification, and SPF assessment. The total flavonoid content of the ethyl acetate, aqueous, and n-hexane fractions of the ethanolic extract of *Centella asiatica* (L.) Urb. leaves was found to be 2.53 mg QE/g, 1.24 mg QE/g, and 0.60 mg QE/g, respectively. The SPF values of the ethanolic leaf extract at concentrations of 100, 200, 300, 400, and 500 ppm were classified as providing minimal, moderate, maximum, maximum, and ultra protection, respectively. These results indicate that *Centella asiatica* (L.) Urb. leaves have significant potential as a natural source of antioxidants and as a sunscreen agent capable of offering effective protection against ultraviolet (UV) radiation.

Keywords: Total flavonoids, fractionation, *Centella asiatica* (L.) Urb., sun protection factor (SPF).

Introduction

The skin is the largest organ of the human body, covering approximately 1.5–2.0 m² of surface area. It serves both protective and aesthetic functions [1]. Healthy skin is typically characterized by even tone, adequate moisture, elasticity, and smooth texture [2,3]. Functionally, the skin acts as a physical barrier against various environmental insults, including ultraviolet (UV) radiation from sunlight [4]. Solar radiation that reaches the Earth's surface comprises ultraviolet (200–400 nm), visible (400–760 nm), and infrared (>760 nm) wavelengths [5,6]. Among these, UV radiation is subdivided into UV-A (320–400 nm), which penetrates the dermis, and UV-B (290–320 nm), which primarily affects the epidermis [7,8].

Although limited UV exposure plays a beneficial role in vitamin D synthesis [9], chronic and excessive exposure can induce structural damage to the skin. Acute responses include erythema, hyperpigmentation, and photosensitivity, whereas prolonged exposure contributes to premature aging, DNA damage, and increased risk of skin malignancies such as actinic keratosis and melanoma [10–12].

To mitigate the harmful effects of UV radiation, the topical application of sunscreen products is widely recommended. Sunscreens are formulations designed to protect the skin by absorbing, reflecting, or scattering UV rays [13]. Ideal sunscreens should effectively block UV-B radiation (290–320 nm) while allowing some degree of UV-A transmission (320–400 nm) to prevent complete inhibition of vitamin D synthesis [14,15]. However, many conventional sunscreens are based on synthetic compounds, which may cause adverse dermatological reactions, including irritation and allergic contact dermatitis [16,17].

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This has led to increased interest in natural compounds with UV-protective potential as safer alternatives [18].

Plant-derived secondary metabolites, particularly flavonoids, have been explored for their photoprotective properties. These compounds possess chromophore structures capable of absorbing UV radiation and converting it into less harmful thermal energy, thereby exhibiting sunscreen activity [19,20]. Moreover, flavonoids may contribute to enhancing the protective barrier of the skin and are suitable candidates for incorporation into topical formulations [21].

Centella asiatica (L.) Urb., a medicinal plant traditionally used in Asian countries, has been reported to exhibit various dermatological benefits, including wound healing, moisturization, and anti-photoaging effects. These effects are attributed to its bioactive constituents such as flavonoids, terpenoids, and tannins [22]. Notably, the flavonoid content in *Centella asiatica* (L.) Urb. may contribute to its UV-absorbing capabilities, reinforcing its potential as a natural sunscreen agent. Therefore, this study aims to determine the total flavonoid content and evaluate the Sun Protection Factor (SPF) of *Centella asiatica* (L.) Urb. leaf extracts obtained using different solvents.

Materials and Methods

The materials and reagents used in this study included the leaves of *Centella asiatica* (L.) Urb., 70% ethanol, absolute ethanol, n-hexane, ethyl acetate, distilled water, ferric chloride ($FeCl_3$), magnesium powder, hydrochloric acid (HCl), Mayer's reagent, Liebermann-Burchard reagent, Bouchardat's reagent, chloroform, methanol, sodium acetate, aluminum chloride ($AlCl_3$), and quercetin standard. All chemicals were of analytical grade and obtained from reputable suppliers.

The instruments used included a rotary evaporator (Heidolph, Germany), UV-Visible spectrophotometer (Shimadzu UV-1800, Japan), silica gel GF254 TLC plates (Merck, Germany), a TLC developing chamber, and preparative TLC equipmen

Preparation and Characterization of Plant Extract

Dried leaves of *Centella asiatica* (L.) Urb. (500 g) were extracted using maceration with 70% ethanol as the solvent. The plant material was immersed completely and allowed to stand at room temperature for 5 days with occasional stirring. After filtration and manual pressing of the marc, the residue was subjected to a second maceration using fresh solvent under identical conditions. The combined filtrates from both cycles were concentrated under reduced pressure at 45–50 °C using a rotary evaporator to obtain a viscous crude ethanolic extract [23].

The plant simplicia and resulting ethanolic extract were characterized through physicochemical analyses, including moisture content, ethanol-soluble extractive value, water-soluble extractive value, total ash content, and acid-insoluble ash content. All procedures were conducted according to standard pharmacopeial protocols [24].

Phytochemical Screening and Fractionation

The phytochemical screening of *Centella asiatica* (L.) Urb. leaf extracts was performed to identify the presence of flavonoids, tannins, saponins, alkaloids, and terpenoids using standard phytochemical techniques and specific reagents.

- Flavonoids were detected by the alkaline reagent test, where a few drops of 10% sodium hydroxide (NaOH) solution were added to the extract. The formation of an intense yellow color, which turned colorless upon the addition of dilute hydrochloric acid, indicated the presence of flavonoids.
- Tannins were identified by adding 1% ferric chloride ($FeCl_3$) solution to the extract. A positive result was indicated by the development of a blue-black or greenish-black coloration.
- Saponins were screened using the froth test. The extract was vigorously shaken with distilled water, and the formation of a stable, persistent froth indicated the presence of saponins.
- Alkaloids were detected using Wagner's reagent (iodine in potassium iodide solution). The formation of a reddish-brown precipitate upon addition of the reagent confirmed the presence of alkaloids.
- Terpenoids were determined using the Salkowski test, where chloroform was added to the extract, followed by the addition of concentrated sulfuric acid (H_2SO_4). The appearance of a reddish-brown interface indicated the presence of terpenoids.

All tests were conducted in triplicate to confirm the reproducibility of results, following established procedures in phytochemical analysis [25].

For fractionation, 20 grams of the ethanolic extract were dissolved in 100 mL of distilled water and placed in a separating funnel. The first fractionation step involved the addition of n-hexane, followed by vigorous shaking. After allowing the mixture to stand, two phases were formed: the n-hexane and aqueous phases. This process was repeated three times to ensure complete extraction. The n-hexane-soluble fraction was then separated and evaporated under reduced pressure to obtain the n-hexane fraction. The n-hexane-insoluble fraction was further fractionated with an equal volume (1:1) of ethyl acetate. This fractionation was repeated twice, and the ethyl acetate-soluble fraction was collected and evaporated to yield the ethyl acetate fraction. Finally, the ethyl acetate-insoluble fraction was collected and evaporated to obtain the water-soluble fraction [26,27].

Quantitative and Qualitative Analysis of Flavonoids in *Centella Asiatica* (L.) Urb. Leaf Extract

The qualitative and quantitative analysis of flavonoids in the ethanol extract of *Centella asiatica* (L.) Urb. leaves was conducted through several steps. First, Thin Layer Chromatography (TLC) was performed using a silica gel GF254 plate (8 cm × 1 cm) as the stationary phase. The concentrated extract, dissolved in 70% ethanol, was applied as a spot 1 cm from the bottom edge and allowed to air dry. The plate was developed in a chamber pre-saturated with a mobile phase consisting of chloroform and ethyl acetate (3:7). Upon reaching the solvent front, the plate was removed, dried, and observed under UV light at 366 nm, then sprayed with 5% $AlCl_3$. The resulting spots were scraped and dissolved in methanol for further analysis [28].

For identification, the isolates obtained from TLC were centrifuged and dissolved in methanol. The UV-Vis absorption spectrum was measured at 366 nm and compared with that of quercetin as the reference standard to confirm the presence of flavonoid compounds [29].

To determine the maximum wavelength of quercetin, the absorbance of a quercetin solution was recorded across the 370–450 nm range. The peak absorbance was selected for subsequent measurements [30]. Next, a 100 ppm quercetin working solution was reacted with 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M sodium acetate, and 2.8 mL distilled water. Absorbance was recorded at 3-minute intervals over 400–800 nm until a stable reading was achieved, establishing the optimum operating time [31].

A standard calibration curve was constructed using quercetin concentrations of 2, 4, 6, 8, 10, and 12 ppm, each treated with the same reagents and measured after the determined operating time [32]. Subsequently, total flavonoid content in the ethyl acetate, water, and n-hexane fractions was quantified. Each fraction (25 mg) was dissolved in ethanol (25 mL) to make a 1000 ppm stock. A 1 mL aliquot of each was treated with $AlCl_3$, sodium acetate, and distilled water as described above. After standing for the operating time, the absorbance was measured using UV-Vis spectrophotometry at the previously established maximum wavelength [33].

In Vitro Evaluation of Sun Protection Factor (SPF) of the Extract

The sunscreen potential of *Centella asiatica* (L.) Urb. leaf ethanol extract was evaluated using in vitro SPF analysis. A stock solution of 1000 ppm was prepared and diluted to 100, 200, 300, 400, and 500 ppm. Ethanol was used as the solvent and blank for spectrophotometric calibration. Absorbance was measured at 5 nm intervals over the 290–320 nm range using a UV-Vis spectrophotometer [34].
The SPF values were calculated according to the Mansur equation:

$$\mathrm{SPF} = \frac{\Sigma\,(I_\lambda \,.\, E_\lambda \,.\, A_\lambda)}{\Sigma\,(I_\lambda \,.\, E_\lambda)}$$

Where:

- I_λ = Intensity of UV radiation at wavelength λ (290–320 nm)
- E_λ = Erythemal efficiency factor at wavelength λ, reflecting the sensitivity of skin to UV radiation.
- A_λ = Absorbance of the *Centella asiatica* (L.) Urb. extract at wavelength λ.

The SPF calculation was performed based on the absorption data recorded for each concentration, following standard protocols outlined in the literature [35].

Results and Discussion

Preparation and Phytochemical Characterization of *Centella Asiatica* Extract

Pharmacognostic characterization of *Centella asiatica* (L.) Urb. leaf simplicia was conducted to ensure the quality and purity of the raw material. The results are presented in Table 1.

Table 1. Pharmacognostic characterization of *Centella asiatica* (L.) Urb. leaf simplicia

	Parameter	Observed Value (%)	Reference Standard (%)
1	Water content	6.33	<10
2	Water-soluble	12.22	>6
3	Extractive content	19.6	>9.5
4	Ethanol-soluble extractive content	14.31	<19
5	Total ash content Acid-insoluble ash content	1.29	<4.5

Reference Standard [46].

The water content of *Centella asiatica* (L.) Urb. leaf simplicia powder was determined to be 6.33%, which is within the acceptable range (<10%). Excess moisture in simplicia may promote microbial, fungal, or insect growth, leading to the degradation of active ingredients [36]. The water-soluble extractive content of the leaf powder was found to be 12.22%, while the ethanol-soluble extractive content was 19.59%. These findings suggest that the ethanol-soluble compounds in *Centella asiatica* (L.) Urb. leaves are more abundant than the water-soluble ones, likely due to the greater solubility of certain chemical compounds in ethanol compared to water [37]. The total ash content was measured at 14.31%, which falls within the standard limit (<19%). Acid-insoluble ash content, which reflects potential contamination by minerals or metals, was found to be 1.29%. This value indicates the presence of minimal external contaminants, such as sand or silicate soil particles [38].

Phytochemical screening was carried out to identify the major groups of secondary metabolites in the ethanol extract of *Centella asiatica* (L.) Urb. leaves. The results are summarized in Table 2.

Table 2. Phytochemical screening results of *Centella asiatica* (L.) Urb. leaf extract

No	Phytochemical Compound	Result
1	Alkaloids	Positive (+)
2	Flavonoids	Positive (+)
3	Saponin	Positive (+)
4	Tannin	Positive (+)
5	Terpenoids	Negative (-)

Description:
(+) indicates the presence of the tested phytochemical compound.
(–) indicates the absence of the tested phytochemical compound.
The phytochemical screening of *Centella asiatica* (L.) Urb. leaf extract revealed the presence of alkaloids, flavonoids, saponins, and tannins. However, no terpenoid compounds were detected in the extract.

Liquid-liquid fractionation was performed to separate compounds based on polarity using three different solvents: n-hexane (non-polar), ethyl acetate (semi-polar), and water (polar). The results are shown in Table 3.

Table 3. Fractionation results of ethanol extract of *Centella asiatica* (L.) Urb. using different solvents

No	Solvent	Fraction Weight (g)	Yield (%)
1	Ethyl acetate	5.22	26.1
2	*n*-hexane	4.3	21.5
3	Water	17.81	89.05

Note: Fractionation was performed from 20 g of ethanol extract of *Centella asiatica* (L.) Urb. leaves.

Fractionation is a method used to separate compounds based on their polarity. The number and type of compounds separated will vary across fractions, with polar compounds being extracted into polar

solvents, non-polar compounds into non-polar solvents, and semi-polar compounds into semi-polar solvents. In this study, liquid-liquid fractionation was employed to separate the compounds in the extract [39]. The results of the fractionation show that the water fraction had the highest yield, indicating that the majority of the compounds in *Centella asiatica* (L.) Urb. leaves are polar. The yield differences observed among the solvents are due to their varying abilities to extract specific compounds from the leaves [40].

Quantitative and Qualitative Analysis of Flavonoids in *Centella asiatica* (L.) Urb. Leaf Extract

The phytochemical constituents in each fraction of *Centella asiatica* (L.) Urb. leaf extract were identified using Thin Layer Chromatography (TLC). The results are summarized in Table 4. In this analysis, the retention factor (Rf) values were calculated based on the distance traveled by each compound relative to the eluent front. The chromatographic separation was carried out using a chloroform : ethyl acetate (3:7) mobile phase. After development, the TLC plates were observed under UV light at 366 nm and subsequently sprayed with 5% aluminum chloride ($AlCl_3$) solution to enhance the visualization of flavonoid compounds. The ethyl acetate fraction and the quercetin standard showed detectable spots, which were identified as flavonoids based on their fluorescence characteristics and Rf values.

Table 4. Identification results of thin layer chromatography test fractions

Sample	Spot No.	Stain Distance (cm)	Eluent Distance (cm)	Rf Value	Spot Color (UV 254 nm)	Identified Compound
Water Fraction	-	-	-	-	-	-
Ethyl acetate fraction	1	3.8	4.5	0.84	Black	Flavonoids
	2	3.2	4.5	0.71	Black	Flavonoids
	3	2.6	4.5	0.57	Black	Flavonoids
	4	1.8	4.5	0.4	Black	Flavonoids
	5	1.5	4.5	0.33	Black	Flavonoids
n-hexane fraction	-	-	-	-	-	-
Quercetin (standard)	1	3.9	4.5	0.86	Black	Flavonoids

In the TLC analysis, five distinct spots were observed in the ethyl acetate fraction, all of which were identified as flavonoids based on their color and Rf values. No spots were detected in the water and n-hexane fractions, indicating the absence of detectable flavonoid compounds in these extracts. A single spot was observed in the quercetin standard, with an Rf value of 0.86, which was consistent with the flavonoid profile observed in the ethyl acetate fraction. The Rf values for the spots in the ethyl acetate fraction were as follows: 0.84, 0.71, 0.57, 0.40, and 0.33. These values were similar to those of the quercetin standard (Rf = 0.86), supporting the identification of flavonoids in the ethyl acetate fraction.

The UV-Vis spectrophotometric analysis of the flavonoid isolate from the ethyl acetate fraction of *Centella asiatica* (L.) Urb. leaves, obtained through preparative Thin Layer Chromatography (TLC), is presented in Figure 1. The spectrum was recorded in the wavelength range of 200–400 nm.

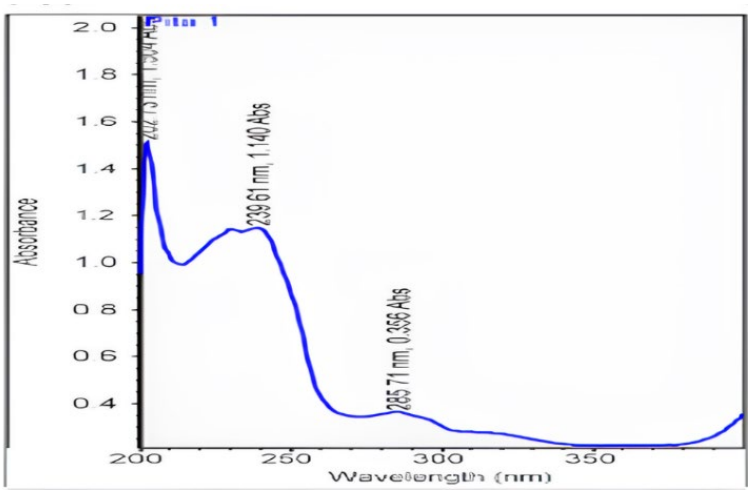

Figure 1. UV-Vis spectrum of ethyl acetate fraction isolate at wavelength of 200-400 nm

For comparison, the UV-Vis spectrum of the quercetin comparator is shown in **Figure 2**, recorded in the wavelength range of 400–800 nm.

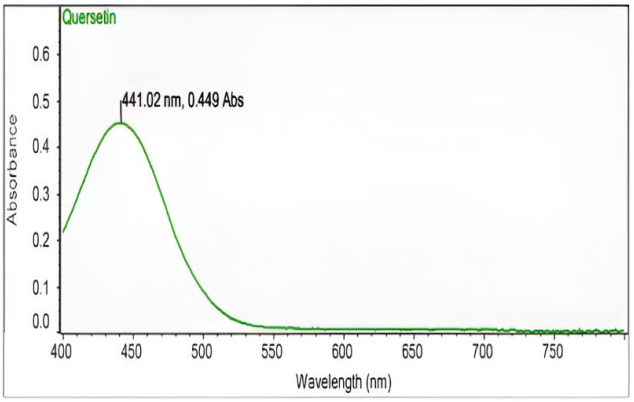

Figure 2. UV-Vis spectra of quercetiin comparator at wavelength of 400-800 nm

The UV-Vis spectrum of the ethyl acetate fraction isolate exhibited a peak at 285 nm, while the quercetin comparator showed a peak at 441 nm. These results suggest that the ethyl acetate fraction isolate contains flavonoid compounds from the flavonol group.

The maximum absorption wavelength of quercetin in ethanol p.a. was determined using UV-Vis spectrophotometry within the wavelength range of 400–800 nm. The analysis showed that the standard quercetin solution exhibited its maximum absorbance at 441 nm, indicating the optimal wavelength for detection. The UV-Vis absorption spectrum of quercetin is shown in Figure 3.

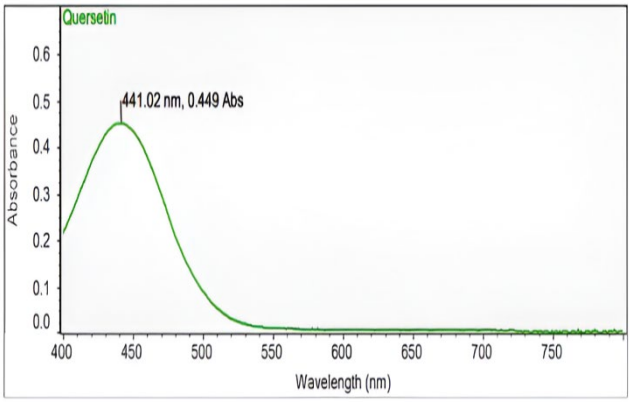

Figure 3. UV-Vis absorption spectrum of quercetin showing maximum absorbance at 441 nm

The calibration curve for the quercetin standard solution was constructed by measuring the absorbance at a wavelength of 441 nm. The absorbance values increased proportionally with concentration, indicating a linear relationship suitable for quantitative analysis. The data used to construct the curve can be seen in Table 5. The resulting linear regression equation was $y = 0.0697x + 0.0034$, with a correlation coefficient of $R^2 = 0.993$, demonstrating a strong correlation between quercetin concentration and absorbance at this wavelength. This calibration curve was then used as the basis for determining the total flavonoid content in the various extract fractions.

Table 5. Calibration curve data of quercetin standard solution at 441 nm

Concentration (ppm)	Absorbance
4	0.282
6	0.421
8	0.576
10	0.672
12	0.854

The data clearly demonstrate a linear increase in absorbance with increasing concentration of quercetin, supporting the reliability of the calibration curve for flavonoid quantification in the samples.

The total flavonoid content in each solvent fraction was determined using UV-Vis spectrophotometry, with quantification based on the quercetin calibration curve measured at 441 nm. This method allowed for accurate estimation of flavonoid levels across different polarities of the extract. The results obtained from the ethyl acetate, n-hexane, and water fractions are summarized in Table 6.

Table 6. Total flavonoid content in different solvent fractions of *Centella asiatica* (L.) Urb. leaves

Fraction	Replication	Concentration (ppm)	Absorbance	Flavonoid content (mg QE/g)	% Flavonoid Content
Ethyl acetate	I	100	0.444	2.52852	0.25 %
	II	100	0.445	2.53428	
	III	100	0.444	2.52852	
Water	I	200	0.438	1.24704	0.12 %
	II	200	0,435	1.23844	
	III	200	0.435	1.23844	
n-Hexane	I	300	0.323	0.60520	0.059 %
	II	300	0.316	0.59200	
	III	300	0.319	0.59750	

The UV-Vis spectrophotometric method was used to measure the total amount of flavonoids. The standard solution used to compare total flavonoid compounds had its maximum wavelength recorded before it was put to the test. What was used as a comparison was quercetin. The test results showed that the standard solution in ethanol p.a. absorbed light best at a wavelength of 441 nm, which is in the range of colors that can be seen [41].

By finding the maximum wavelength, the wavelength at which the compound being tested gives the best absorbance of immersion can be found. The test is very sensitive and linear when the compound gives the best absorbance. This means that a slight change in the concentration of the compound will cause a significant change in the absorbance. To find the operating time of a compound, first get its maximum wavelength from the reference standard solution [42]. The operating time tells you how long to measure the compound when its absorbance is stable. Stabilizing the absorbance takes 30 minutes, so finding the proper working time is crucial.

First, the reference solution for total flavonoid analysis was used to measure absorbance. We measured absorbance using a UV-Vis spectrophotometer with a maximum absorbance of 441 nm set before the tests. A linear regression equation y = 0.0697x + 0.0034 was found by measuring the absorption of total flavonoids to find the calibration curve of quercetin at a wavelength of 441 nm. With a correlation coefficient of 0.993, the standard solution of flavonoid compounds showed a straight-line link between absorbance and concentration. The linear regression equation is linear if the number (r) is one.

In this study, the total flavonoid content obtained in each of the fractionation results of ethyl acetate 100 ppm, water 200 ppm, and *n*-hexane 300 ppm is equal to 2.53044 mg QE/g fraction, 1.24130 mg QE/g fraction, and 0.5982 mg QE/g fraction. The concentration used to determine total flavonoid levels in each sample is different because the water and *n*-hexane fraction samples with the same concentration of 100 ppm obtained absorbance below the absorbance of the standard curve [43,44]. The difference in concentrations (100, 200, and 300 ppm) was a necessary adjustment to ensure that each sample's absorbance fell within the linear range of the calibration curve. Attempts to standardize all fractions at 300 ppm were constrained by solubility and absorbance limitations in certain fractions (e.g., ethyl acetate produced excessively high absorbance at this level). Therefore, each concentration was optimized individually to ensure accuracy of flavonoid quantification. However, we acknowledge this limits direct quantitative comparison among fractions.

In Vitro Evaluation of Sun Protection Factor (SPF) of the Extract

The SPF values of *Centella asiatica* (L.) Urb. leaf ethanol extract were determined at five different concentrations (100–500 ppm) using UV-Vis spectrophotometry across the UVB range (290–320 nm). The SPF values increased proportionally with concentration, indicating a dose-dependent photoprotective effect. As shown in Table 7, at 100 ppm, the extract exhibited a minimum level of protection (SPF = 2.53), while at 500 ppm, it demonstrated ultra protection (SPF = 15.21), based on commonly accepted SPF classification standards.

Table 7. Sun protection factor (SPF) value of *Centella asiatica* (L.) Urb. leaves extracts

Sample Solution	Concentration	SPF Value	Category
Centella asiatica (L.) Urb. Leaves Ethanol Extract	100 ppm	2.53	Minimum
	200 ppm	4.89	Medium
	300 ppm	8.01	Maximum
	400 ppm	11.51	Maximum
	500 ppm	15.21	Ultra

The Sun Protection Factor (SPF) is a widely accepted parameter that quantifies the effectiveness of a substance in protecting the skin against ultraviolet (UV)-induced erythema. A higher SPF value corresponds to a greater level of photoprotection, indicating enhanced prevention of UV-induced skin damage and erythema [45].

In this study, the SPF values of *Centella asiatica* (L.) Urb. leaf ethanol extract at concentrations of 100, 200, 300, 400, and 500 ppm were determined using UV-Vis spectrophotometry across the UVB spectrum (290–320 nm). The absorbance data obtained were then applied to the Mansur equation to calculate SPF values.

The resulting SPF values were 2.53, 4.89, 8.01, 11.51, and 15.21 for concentrations of 100, 200, 300, 400, and 500 ppm, respectively. Based on widely used SPF classification guidelines, these correspond to minimum, moderate, maximum, maximum, and ultra protection categories, respectively. The results indicate a concentration-dependent increase in SPF value, supporting the potential use of *Centella asiatica* (L.) Urb. leaf extract as a natural sunscreen agent.

Conclusions

The total flavonoid content in the ethyl acetate, water, and n-hexane fractions of *Centella asiatica* (L.) Urb. ethanolic leaf extract was 2.53044 mg QE/g, 1.24130 mg QE/g, and 0.59820 mg QE/g of fraction, respectively. The Sun Protection Factor (SPF) values of the ethanolic leaf extract at concentrations of 100, 200, 300, 400, and 500 ppm were 2.53 (minimum protection), 4.89 (medium protection), 8.01 (maximum protection), 11.51 (maximum protection), and 15.21 (ultra protection), respectively.

Conflicts of Interest

Authors Declare No Conflict of Interest.

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