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# Research Article A Study on Sun Production Factor and Antioxidant Activity of Senna italica Mill

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# **Abstract**

**Background and Objective:** *Senna italica* is a medicinal herb that the empirical medicine community has extensively used. This study intends to assess the bioactivity of *S. italica* extract as a sunscreen using *in vitro* methods to acquire UV protection factors and antioxidant activity. **Materials and Methods:** In this work, dried *S. italica* leaves were extracted by graduated maceration with n-hexane, ethyl acetate and methanol (80% v/v) to produce an extract. With a UV spectrophotometer, extracts from plant leaves were examined for the sunscreen profile in terms of UVA (320-400 nm) and UVB (290-320 nm) absorption. The extract was evaluated for UV protection using test criteria such as antioxidants and sun protection factor (SPF). **Results:** The findings of sunscreen profiles in absorbing UVB and UVA revealed that hexane, ethyl acetate and methanol extracts of leaves could absorb both UVB and UVA. Leaf methanol extract had the highest UVB and UVA absorption rates. The extract demonstrated the capacity to prevent regular tanning (leaf methanol extract). The SPF characteristics of *S. italica* herbal sample showed that leaf methanol extract at a concentration of 50, 100 and 150 μg/mL gave an ultra-protective effect with SPF values of 13.4, 15.7 and 18.4, respectively. Among the three parameters, the maximum UV protection capacity was observed at 150 μg/mL, with a sun protection factor value of 18.4. The *S. italica* extract demonstrated strong antioxidant activity, with an IC<sub>50</sub> value of 92.58 at 20 μg/mL. **Conclusion:** The findings of this study revealed that methanolic extracts of *S. italica* have the potential to operate as a sunscreen, providing ultra-protection against UVA and UVB rays as well as antioxidant activities.

Key words: Sun protection factor, sunscreen, ultraviolet radiation, antioxidant, Senna italica

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

# **INTRODUCTION**

Solar UV radiation is classified according to wavelength: UVA (315-400 nm), UVB (280-315 nm) and UVC (100-280 nm)<sup>1</sup>. The UVC rays are diffused and diminished by the ozone layer, so they do not reach the ground. The earth's surface receives approximately 90-99% of UVA and 1-10% of UVB radiation. The skin's epidermis acts as a barrier, protecting the body from the outside world<sup>2</sup>. Dalbergia monetaria from the Brazilian Amazon has been identified with a notable SPF value, indicating its potential for sunscreen use<sup>3</sup>. The extract from Hylocereus polyrhizus, is abundant in rutin and phenolic acids, such as gallic and sinapic acid. This extract exhibited absorption of both UVA and UVB rays and displayed antioxidant and photoprotective properties<sup>4</sup>. The UV filters are based on sinapate esters, which offer both high UV photostability and environmental sustainability. The functionalised symmetrically sinapate esters nature-inspired UV filters for next-generation sunscreen formulations5.

Chemical sunscreens are classified according to their level of UV radiation protection. The UV-A absorbers protect against 320-380 nm wavelengths, which include benzophenones, eradicate and avobenzone. The UV-B absorbers protect against wavelengths ranging from 290 to 320 nm. The UVB radiation boosts melanin formation, resulting in a long-lasting tan and thicker epidermis, but it can also induce sunburn. The UVA radiation penetrates deeper into the skin, activating existing melanin for a brief tan while also contributing to long-term skin damage, ageing and the risk of free radical production cancer<sup>6</sup>.

For those with sensitive skin, the development of herbal sunscreen agents provides a safer alternative because they are often well-received and help to reduce the risk of carcinogenesis<sup>7</sup>. Sunscreen products of the 21st century should protect against not just sunburn, erythema and redness, but also guard against cellular damage that significantly contributes to the rising incidence of skin carcinomas and melanomas. While sunscreen products are crucial in preventing skin cancer, their misuse by consumers or the need for high concentrations of chemical filters to achieve maximum protection (SPF 50+) has been linked to the continued rise in skin cancer cases. Since some organic filters and their byproducts survive in the environment, are harmful to aquatic life and may interfere with hormone effects, their use is now prohibited even though they can increase the SPF of sunscreens by blocking a broad spectrum of UV rays<sup>8,9</sup>.

Sunscreen is the best way to shield skin from UV radiation and is the most efficient way to prevent phototoxic damage to the skin, which includes pigmentation, sunburn, ageing of the skin and destruction of collagen<sup>10,11</sup>. Several analytical techniques have been established to assess the antioxidant activity of plant extracts, including the capacity to eliminate radicals, ABTS<sup>12</sup>. Many researchers have reported the strong antioxidant activity of different plant extracts<sup>13-21</sup>. Natural products and extracts of plants with antioxidant qualities offer the potential for treating and preventing UV-induced skin damage.

The CGA from *Coffea arabica* beans inhibited intracellular ROS production in UV-stimulated CCRF cells in a dose-dependent manner. Pretreatment of HaCaT cells with a methanol extract of *Juglans regia* L.'s male flower for 0.5 hr before UVB-irradiation decreased ROS generation and lipid peroxidation while restoring antioxidant activity<sup>22</sup>. Muzaffer *et al.*<sup>23</sup> found that cold-pressed perilla oil produced from *Perilla frutescens* reduced UV-induced ROS production in NHDF cells. Because of the plant's widespread use and value as a medicinal plant, various studies have been conducted, primarily on its essential oil, to better understand its therapeutic properties and chemical composition<sup>24</sup>. Crude extracts of *Mentha pulegium* have also been shown to have antioxidant, antimicrobial and insecticidal properties<sup>25,26</sup>.

Several clinical and *in vitro* studies have shown that UV radiation exposure may be directly responsible for several skin diseases, such as aging, dry skin, vasodilation, melanoma and skin cancer<sup>27</sup>. Phenolic compounds are differentiated by an absorption spectrum that filters out UV radiation, hence reducing damaging UV ray penetration into the skin, oxidative stress and DNA damage<sup>28</sup>. Natural polyphenol molecules, which have antioxidant, anti-inflammatory and photoprotective properties, can help protect against the detrimental effects of UV radiation from the sun<sup>29</sup>. The present investigation aims to examine the biological effects of methanolic extracts of *S. italica* from Tirunelveli, India. The antioxidant and sun protection factor capabilities have been investigated for potential medicinal or cosmetic applications.

# **MATERIALS AND METHODS**

**Study area:** The study was conducted from January to December, 2023 at the Department of Botany, Rani Anna Government College for Women, Tirunelveli-8, Tamil Nadu, India.

**Study material:** The plant materials (*S. italica*) were collected from the field of Tirunelveli District of Tamilnadu, India. The collected twenty-five samples of *S. Italica* were first washed under running tap water and air-dried in the shade at room temperature for 10 to 15 days<sup>30</sup>. The dried plant material was finely powdered using a home grinder and sieved through mesh No. 80 and stored in an airtight container till further use. Subsequently, each 50 g of powdered material was macerated in hexane, methanol and ethyl acetate for 3 days, filtered and repeated three times. The resulting supernatant was collected and the solvent evaporated to obtain crude extract and refrigerated at 4°C for further use.

**Sample preparation and crude extract formation:** Each extract, at a concentration of 10 mg in 100 mL of methanol to achieve 100 μg/mL, underwent ultrasonication for 10 min and subsequent filtration through Whatman filter paper. Following this, each extract at a concentration of 50 μg/mL was scanned across the wavelength range of 200 to 400 nm using a UV-visible spectrophotometer (Perkin Elmer Lambda 35 UV-visible spectrophotometer, New Alkapuri, Gotri, Vadodara 390 021, Gujarat, India). All the readings were repeated 3 times at each point. Absorption spectra were measured in a 1 cm quartz cell using the "Spectra Measurement" mode, with a reference cell containing methanol as the pure solvent<sup>31</sup>. The absorption spectra and characteristic peaks of *S. italica* leaf extracts were recorded.

DPPH radical scavenging activity assay: The radical scavenging activities of the plant extracts against 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical (Sigma-Aldrich) were determined by UV spectrophotometry at 517 nm. The following concentrations of the extracts were prepared: 20, 40, 60, 80, 100 and  $200 \mu g/mL$  in methanol (Analar grade). Ascorbic acid was used as the antioxidant standard at the same concentrations: 20, 40, 60, 80, 100 and 200 µg/mL. One milliliter of DPPH solution was added to each sample and the final volume was adjusted with methanol. The negative control was prepared by mixing 1 mL of DPPH with 3 mL of methanol. All samples were then incubated for 30 min at room temperature. The IC<sub>50</sub> (Half Maximal Inhibitory Concentration) was calculated graphically, using a calibration curve, in the linear range by plotting the extract concentration versus the corresponding scavenging effect (RSA (%)), over 30 min. Ascorbic acid was used as a positive antioxidant control. The assay was performed in triplicate<sup>32</sup>:

$$1\% = \frac{\text{Abs control-Abs sample}}{\text{Abs control}} \times 100$$

# UV absorption capacity assessment for antisolar activity:

The UV-visible spectrophotometry, as described was employed to assess the antisolar activity<sup>33</sup>. Preliminary analysis used both water and methanol extracts. Each extract was made at a concentration of 1 mg/mL in its respective solvent and spectrophotometric readings were taken with a UV-visible spectrophotometer at 200 to 450 nm with a 5 nm variation. Every data point had three measurements taken.

**Exploring SPF determination:** The SPF is determined using the method provided by Cefali *et al.*<sup>34</sup>. The dried extract yielded a 1 mg/mL stock solution, which was then adjusted to concentrations of 50, 100 and 150 μg/mL using the appropriate solvents. These solutions were spectrophotometrically measured at 5 nm intervals at wavelengths spanning from 290 to 320 nm and the results were recorded. Every reading was taken in quadruplicate at each position. Determination of sun protection factor by UV-Vis spectophtometry<sup>35</sup>:

SPF = CF 
$$\sum_{290}^{320}$$
 EE  $(\lambda) \times I(\lambda) \times Abs(\lambda)$ 

Where:

CF = Correction factor (10)

 $EE(\lambda)$  = Erythemogenic effect of radiation at wavelength ( $\lambda$ )

 $I(\lambda)$  = Intensity of solar light at wavelength  $(\lambda)$ 

Abs ( $\lambda$ ) = Absorbance of wavelength ( $\lambda$ ) by a solution of the

preparation

The obtained absorbance values were multiplied by the EE ( $\lambda$ ) values, their summation was taken and multiplied by the correction factor 10.

# In vitro determination of Sun Protection Factor (SPF) for

**extracts:** It is critical to explore the sunburn-protective effects of plants known for their therapeutic potential and abundance of phytoconstituents. A sunscreen agent's effectiveness is often measured by its Sun Protection Factor (SPF), which is calculated as the ratio of UV energy required to elicit a minimal erythema dose (MED) on protected skin to that required on unprotected skin<sup>36</sup>:

$$SPF = \frac{Minimal\ erythemal\ dose\ of\ protected\ skin}{Minimal\ erythemal\ dose\ of\ unprotected\ skin}$$

The SPF of various concentrations (50, 100 and 150 µg/mL) was determined using a stock solution of 100 µg/mL concentration. Measurements were conducted at 290-320 with 5 nm intervals, each concentration analysed in triplicate using a 1 cm quartz cell. Methanol served as the blank, repeated three times and the average absorbance value was derived for each extract concentration. This absorbance value was then multiplied by the  $EE(\lambda) \times I$  constant from Table 1, with the sum of these products further multiplied by a correction factor, a constant of 10. Mansur offered a simple mathematical equation as an alternative to Sayre *et al.*<sup>37</sup> *in vitro* approach, which used a UV spectrophotometer. This formula was used to compute SPF.

### Where:

CF = Correction factor (10)

EE ( $\lambda$ ) = Erythrogenic effect of radiation I ( $\lambda$ ) = Solar intensity spectrum Abs

 $\lambda$  = Spectrophotometric absorbance value

The values of  $\mathsf{EE}\,\mathsf{x}\,\mathsf{I}$  are constants. The results were shown in Table 1.

Malsawmtluangi *et al.*<sup>36</sup> statistical analysis was employed to achieve the calculation.

## **RESULTS AND DISCUSSION**

The results obtained from these selected plant extracts demonstrated good UV absorption activity. The extracts

of aerial sections of the plants displayed maximum absorbance at 200 and 250 nm and minimum absorbance at 400 and 450 nm, indicating that the extracts can absorb UV radiation (Table 1). Based on these findings, the study is expanded to include SPF analysis. The aerial parts extracts of S. italica in methanol extracts have shown the highest SPF activity, i.e., 18.4 at 150 Î 1/4 g/mL concentration compared to the remaining plant extracts. The SPF values range from 13.4 to 18.4 across different concentrations. Khazaeli and Mehrabani<sup>38</sup> reported that their studies in Datura moldavica and Viola tricolor plants SPF value. Sutar and Chaudhari<sup>39</sup> found the effectiveness in providing UV protection factor Zingiber officinale extracts. The high SPF value in the S. italica extract is due to its flavonoid compounds, which have protective activity from ultraviolet radiation (Table 2).

The SPF analysis was conducted, to determine the SPF values of the *S. italica* extracts at different wavelengths ranging from 290 to 320 nm at 5 nm intervals (Table 1). The graphical representation also mentioned the SPF values of *S. italica* at 5 nm wavelength intervals (Fig. 1). da Silva *et al.*<sup>40</sup> evaluated the SPF activity of the crude extract of *Pothomorphe umbellata* root and found an SPF value of 21.53, which is compatible with the current findings. According to Khazaeli and Mehrabani<sup>38</sup>, the presence of significant levels of flavonoids and phenolics increases the SPF value in plant extracts such as *D. moldavica* and *V. tricolour*, which have SPF activities of 24.79 and 25.69, respectively.

Table 1: Product function used in the calculation of SPF

Wavelength (nm)	EE (λ) X I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
	1

EE: Erythemal effect spectrum and I: Solar intensity spectrum

Table 2: Category of SPF value effectiveness of *S. italica* extract

Senna italica extract							
50 μg/mL		100 μg/mL		 150 μg/mL			
Abs	Abs×EE×I	Abs	Abs×EE×I	Abs	Abs×EE×I		
2.455	0.036	2.599	0.038	2.789	0.041		
2.033	0.166	2.282	0.186	2.489	0.203		
1.601	0.460	1.835	0.527	2.093	0.601		
1.235	0.404	1.478	0.484	1.763	0.577		
1.011	0.188	1.25	0.233	1.527	0.284		
0.844	0.070	1.092	0.091	1.359	0.113		
0.729	0.013	0.973	0.017	1.261	0.022		
	13.4		15.7		18.4		

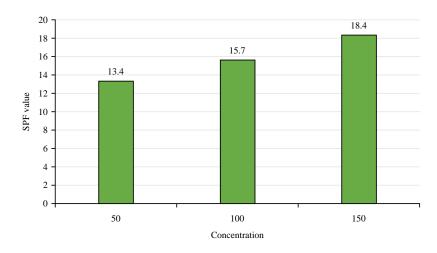


Fig. 1: Graphical representation of SPF activity of S. italica at different concentrations

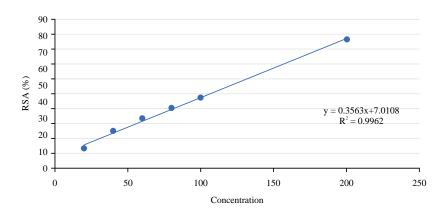


Fig. 2: DPHH radicle scavenging activity of *S. italica* plant extracts

Table 3: Classification of antioxidants based on IC<sub>50</sub> values

IC <sub>50</sub> value (μg/mL)	Antioxidant activity
<50	Very strong
50-100	Strong
101-250	Medium
250-500	Weak
>500	Not active

Table 4: Antioxidant activity of *S. italica* plant extracts

Concentration (μg/mL)	Control	Sample	RSA (%)	IC <sub>50</sub>			
20	0.094	0.083	11.70213	36.45±0.1			
40	0.094	0.073	22.34043	92.58±0.1			
60	0.094	0.066	29.78723	148.72±0.2			
80	0.094	0.06	36.17021	$204.85 \pm 0.3$			
100	0.094	0.054	42.55319	260.98±0.4			
200	0.094	0.021	77.65957	541.64±0.1			

RSA (%): Percentage of radical scavenging activity, Mean±SD

The antioxidant method quantitatively determined the DPPH activity, expressed as  $IC_{50}$ , which is the concentration needed to inhibit 50% of DPPH free radicals<sup>41</sup>. The antioxidant activity extract was taken at a different concentration. It was observed that the higher the extract concentration, the higher

the percentage value of inhibition shown in (Table 3), which revealed that the methanolic aerial extract of *S. italica* exhibited significant antioxidant activity. The *S. italica* extract exhibited strong antioxidant activity, with an  $IC_{50}$  value of 92.58 µg/mL at a 20 µg/mL concentration (Table 4).

Researchers found their studies of plant extracts i.e., *Durio zibethinus, Andrographis paniculata* and red and purple rosella flower petals extract (*Hibiscus sabdariffa*) flavonoid compounds can activate antioxidant enzymes, catalyze metal chelates, transfer-free electrons, reduce alphatocopherol radical, inhibit oxidase and acts as an immunomodulator<sup>42-44</sup>.

According to Mishra et al.45, flavonoids and phenolics exhibit outstanding antioxidant and photoprotective effects. To develop topical sunscreen formulations from specific plant species, it is necessary to screen for phenolics and flavonoids. The results demonstrated the antioxidant activity of *S. italica* extract, which is extremely potent (Fig. 2). A recent study focused on the usage of antioxidants in sunscreens for photoprotection. Plants rich in antioxidants may be a new source for treating and preventing UV-induced allergies, tans and other conditions. The D. metel plant includes phenolic and flavonoid components with free radical scavenging activity, which are linked to antioxidant qualities<sup>46</sup>. To create topical sunscreen formulations from selected plant species, it's important to screen for their phenolics and flavonoids. The results proved the antioxidant power of S. italica extract, which has a very strong antioxidant power.

Recent study has focused on the use of antioxidants in sunscreen for photoprotection. Plants high in antioxidants could be a new source for treating and preventing UV-induced allergies, tans and other disorders. Experiments on S. italica revealed the existence of phenolic chemicals, which have been shown to have antioxidant characteristics. Rasheed et al.47 revealed that Alpinia galanga is used as a primary ingredient in many sunscreen products due to its UV-protective properties and capacity to improve the performance of standard sunscreens. Napagoda et al.48 discovered that Leucas zeylanica and Ophiorrhiza mungos exhibit strong UV absorption in the 260-350 nm range, indicating their usefulness as broad-spectrum sunscreens. Ophiorrhiza mungos extract remained photosensitive after 21 days of direct sunshine exposure. A phytochemical examination of the plant identified flavonoids, terpenoids, saponins and reducing sugars.

# **CONCLUSION**

The preliminary results of this investigation show that the methanol extract of *S. italica* has a strong sun protection effect. This extract can be added to other sunscreen compositions to improve the SPF. This study revealed plant extracts' UV-absorbing ability and sun protection capabilities,

implying that they have the potential to be helpful, cost-effective and widely available sun protection components. The findings of the antioxidant activity test were obtained using the DPPH method. *Senna italica* contains essential oils, vitamin E, hexadecanoic acid, (Z)-9-Hydroxy-2,4-dimethyl-non-7-enoic acid lactone, 1-Butanol, 2-Methyl-, acetate and others. In aerial regions, the extract absorbs more UV rays and has antioxidant properties. In the future, these botanical extracts, either alone or in combination with other additives, could be used to create photoprotective formulations such as creams and lotions with higher SPF levels, as well as to determine the action mechanism of the active compounds found in *S. italica* that protect against UV radiation.

# SIGNIFICANCE STATEMENT

Growing concern over synthetic sunscreens has increased the demand for natural alternatives. This study focused on *S. Italica*, a plant with UV-protective properties. The study discovered that the plant's methanol extract had a high SPF rating of 18.4 and powerful antioxidant activities. The major goal was to assess *S. Italica* extracts as natural sunscreen components and discover the bioactive chemicals that are responsible for their protective properties. It has a strong capacity to protect the skin from UV radiation, making it an ideal ingredient in sunscreen creams. The extract also has a strong antioxidant activity and contains bioactive components that are known to protect against UV rays. This study sets the framework for future research on the creation of natural sunscreens and skincare.

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