



Photoprotective Potential of Two Medicinal Plants: An *In-vitro* Evaluation of Sun Protection Factor and UV Absorption

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Abstract: Background: Ultraviolet (UV) radiation is an important environmental factor contributing to skin damage and photoaging. The search for naturally derived photoprotective agents has increased interest in medicinal plants as potential sources of UV-absorbing compounds. *Salvia haematodes* and *Phlogacanthus thyrsoiflorus* are medicinal plants with established traditional and pharmacological importance, but their potential as sources of natural photoprotective agents requires further investigation. **Aim:** This study aimed to evaluate the in vitro sun protection factor (SPF) of extracts and solvent fractions of *S. haematodes* root and *P. thyrsoiflorus* flower and to identify fractions with promising photoprotective potential. **Materials and Methods:** Root material of *S. haematodes* and flower material of *P. thyrsoiflorus* were extracted using methanol and subsequently subjected to solvent fractionation. The SPF of the prepared extracts and fractions was evaluated using UV-visible spectrophotometry at a concentration of 100 µg/mL. Absorbance was measured within the UVB wavelength range of 290-320 nm at 5-nm intervals. Methanol was used as the blank, and measurements were performed in triplicate. SPF values were calculated using the Mansur spectrophotometric equation with a correction factor of 10. **Results:** The tested extracts and fractions demonstrated varying degrees of UVB-absorbing activity. Among the preparations evaluated, the chloroform and ethyl acetate fractions of *S. haematodes* root showed the highest SPF values, 10.18 ± 0.12 and 9.63 ± 0.08 , respectively, at 100 µg/mL. In comparison, the methanol and ethyl acetate extracts of *P. thyrsoiflorus* flower showed SPF values of 5.82 ± 0.05 and 4.16 ± 0.04 , respectively. The results indicate comparatively greater photoprotective potential for the tested *S. haematodes* root fractions. **Conclusion:** The findings demonstrate that selected solvent fractions of *S. haematodes* root possess appreciable *in-vitro* SPF activity, with the chloroform and ethyl acetate fractions showing the highest values among the tested samples. These findings suggest that *S. haematodes* may serve as a promising botanical source of photoprotective constituents. Further phytochemical investigation and formulation-based studies are needed to determine the suitability of these fractions for developing plant-derived photoprotective preparations.

Keywords: *Salvia haematodes*, *Phlogacanthus thyrsoiflorus*, sun protection factor, photoprotection, UV absorption, medicinal plants.

INTRODUCTION

Ultraviolet (UV) radiation from sunlight is one of the major environmental factors responsible for adverse effects on the skin. Repeated exposure to UV radiation can promote oxidative stress and molecular changes in skin cells, contributing to photoaging, inflammation, pigmentation, loss of skin elasticity and, in severe cases, photocarcinogenesis (Gromkowska-Kępa KJ et al, 2021). UV radiation reaching the earth's surface is composed mainly of UVA (320-400 nm) and UVB (280-320 nm) (Al-Sadek, T., and Yusuf, N., 2024). UVA penetrates relatively deeply into the skin and contributes substantially to chronic photoaging, whereas UVB has greater erythemogenic activity and is primarily responsible for sunburn and other acute photobiological effects (Brar, G., et al., 2025). Therefore, limiting excessive exposure to both UVA and UVB radiation is an important strategy for protecting the skin from solar-induced damage.

The development and use of sunscreen preparations represent important approaches to reducing the harmful effects of solar radiation. Sunscreen products contain organic and/or inorganic UV filters that absorb, reflect or scatter UV radiation (Thy L.T.M., et al., 2025). Combinations of different UV filters are commonly used to provide broader protection across the solar UV spectrum. However, concerns regarding the safety, tolerability and long-term use of certain synthetic sunscreen ingredients have encouraged continuing research into alternative sources of photoprotective substances. This has increased scientific interest in naturally derived compounds and plant extracts that may provide UV-absorbing activity and could potentially be incorporated into cosmetic and photoprotective preparations (Vilela et al., 2011; Sambandan and Ratner, 2011).

Medicinal plants are particularly attractive sources for the investigation of natural photoprotective agents because they contain chemically diverse secondary metabolites. Phenolic compounds, flavonoids and other plant constituents may contribute to UV absorption and protection against oxidative processes associated with solar exposure (Fonseca, M., et al., 2023). Consequently, extracts and fractions obtained from medicinal plants have increasingly been investigated as potential sources of ingredients for cosmetic and photoprotective applications. Previous investigations have demonstrated that several botanical materials can exhibit measurable UV-absorbing and sunscreen-related activity, supporting their preliminary evaluation as alternatives or complementary ingredients to conventional sunscreen agents (Mishra et al., 2012; Santhanam et al., 2013).

The sun protection factor (SPF) is an important parameter for assessing the relative degree of protection against UVB-induced erythema. Spectrophotometric determination of SPF provides a practical in-vitro screening method for evaluating the UVB-absorbing potential of plant extracts and their solvent fractions (Mandol, A., et al., 2026). In this approach, the absorbance of a test sample is measured over the UVB region, generally between 290 and 320 nm, and the SPF is calculated using an established spectrophotometric equation. Although in-vitro SPF assessment does not replace clinical evaluation of a finished sunscreen formulation, it is useful for preliminary identification of plant materials with promising photoprotective properties (Mishra et al., 2012; Santhanam et al., 2013).

Salvia haematodes is a medicinal plant belonging to the family Lamiaceae and has been investigated for its phytochemical composition and diverse biological activities. Previous studies have reported biologically active extracts and fractions of *S. haematodes*,

including antioxidant and enzyme-inhibitory activities, indicating the presence of potentially valuable secondary metabolites (Shawwal, M., et al., 2017; Tundis, R., et al., 2022). However, compared with these reported biological activities, information concerning the photoprotective and SPF potential of its root extracts and solvent fractions remains limited. This provides a rationale for investigating its potential as a botanical source of UV-protective constituents.

Phlogacanthus thyrsiflorus Nees is another medicinal plant of pharmacological interest. Its different parts have been investigated for various biological and phytochemical properties, but its potential application as a source of natural photoprotective material has received comparatively limited attention (Gupta, P.C., et al., 2024). Thus, investigation of its flower extract for in-vitro SPF activity may provide useful preliminary information regarding its potential relevance to natural photoprotective research.

In the present study, extracts and solvent fractions prepared from the root of *S. haematodes* and the flower of *P. thyrsiflorus* were evaluated for their *in-vitro* sun protection factor using a UV-visible spectrophotometric method. The study specifically aimed to compare the SPF values of the different extracts and solvent fractions and to identify preparations exhibiting comparatively higher UVB-absorbing and photoprotective potential. The findings may provide a preliminary scientific basis for further phytochemical investigation and development of plant-derived photoprotective formulations.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used in the study were of analytical grade. Methanol and other solvents used for extraction and fractionation were obtained from Sigma-Aldrich (USA). The solvents used for extraction and liquid-liquid partitioning were purified by distillation prior to use. Methanol was also used as the solvent and blank for spectrophotometric SPF determination.

Preparation of Plant Material and Extraction

The extraction and fractionation of the plant materials, including the roots of *S. haematodes* Linn. and the flowers of *P. thyrsiflorus* Nees, were carried out at the Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The roots of *S. haematodes* and flowers of *P. thyrsiflorus* were identified by a taxonomist at the Bangladesh National Herbarium (BNH), Mirpur, Dhaka, Bangladesh. The accession numbers for *S. haematodes* root and *P. thyrsiflorus* flower were 42993 and 42992, respectively.

The roots of *S. haematodes* and flowers of *P. thyrsiflorus* were dried and ground into fine powders using a Wiley mill. The powdered plant materials were subjected separately to methanolic extraction using an ultrasound-assisted extraction technique, following the procedures reported by Das et al., 2015; Khursheed, T., et al., 2024. The

extracts were filtered, concentrated under reduced pressure at 40°C using a rotary evaporator, and then freeze-dried to obtain the crude methanolic extracts.

Each crude methanolic extract was reconstituted in methanol and subsequently subjected to liquid-liquid partitioning using solvents of increasing polarity. Sequential partitioning was performed with n-hexane, chloroform, ethyl acetate, and n-butanol. The resulting solvent fractions were concentrated under reduced pressure and lyophilized. The dried fractions were individually stored at -20°C until further analysis.

***In-vitro* Sun Protection Factor Determination**

The *in-vitro* SPF of the prepared extracts and solvent fractions was determined by a UV-visible spectrophotometric method based on the Mansur equation (Mishra et al., 2012; Santhanam et al., 2013).

Each test sample was prepared at a concentration of 100 µg/mL in methanol. The absorbance of each sample was measured using a UV-visible spectrophotometer at wavelengths ranging from 290 to 320 nm at 5-nm intervals. Methanol was used as the blank (Karupasamy, D., and Palayian, L., 2025). All measurements were performed in triplicate.

The SPF was calculated according to the following equation:

$$\text{SPF} = \text{CF} \times \sum [\text{EE}(\lambda) \times \text{I}(\lambda) \times \text{Abs}(\lambda)]$$

where $\text{EE}(\lambda)$ represents the erythemal effect spectrum, $\text{I}(\lambda)$ represents the solar intensity spectrum, $\text{EE}(\lambda) \times \text{I}(\lambda)$ represents the normalized erythemal effect spectrum-solar intensity values, $\text{Abs}(\lambda)$ represents the measured absorbance of the sample at wavelength λ , and CF represents the correction factor, which was set at 10 (Cefali, L. C., et al., 2019).

The normalized values of $\text{EE}(\lambda) \times \text{I}(\lambda)$ used for SPF calculation were as follows:

Wavelength (λ nm)	EE x I (normalized)
290	0.051
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

UV Absorption Spectral Analysis

The UV absorption spectra of the test samples were recorded using a UV-visible spectrophotometer over the wavelength range of 200-400 nm. Each sample was prepared at a concentration of 100 µg/mL in methanol and analyzed using a 1 cm quartz cuvette (Binks, B. P., et al., 2017). The UV absorption profiles of the test samples were compared with that of epigallocatechin gallate (EGCG) prepared at the same concentration, following the procedure reported by Patil et al. 2010; Shukla, R., et al., 2026.

Experimental Replication

All spectrophotometric measurements were performed in triplicate, and the SPF values were expressed as mean $\pm$ standard deviation.

RESULTS AND DISCUSSION

In-vitro Sun Protection Factor (SPF)

The *in-vitro* SPF values of the extracts and solvent fractions of *S. haematodes* root and *P. thyrsoiflorus* flower were determined spectrophotometrically at a concentration of 100 $\mu\text{g/mL}$. The absorbance measurements obtained in the UVB region were used to calculate SPF values according to the Mansur spectrophotometric method. All measurements were performed in triplicate.

The tested samples demonstrated considerable variation in SPF activity. Among the fractions obtained from *S. haematodes* root, the chloroform fraction exhibited the highest SPF value (10.18 ± 0.12), followed by the ethyl acetate fraction (9.63 ± 0.08). The methanol extract obtained after solvent fractionation, n-hexane fraction, methanol fraction and ethyl acetate extract after hexane and dichloromethane partitioning showed SPF values of 3.00, 2.36, 3.34 and 4.13, respectively. These findings indicate that the chloroform and ethyl acetate fractions contained constituents with comparatively greater UVB-absorbing activity than the other tested fractions of *S. haematodes* root.

For *P. thyrsoiflorus* flower, the methanol extract obtained after hexane, dichloromethane and ethyl acetate extraction showed an SPF value of 5.83, whereas the direct methanol extract and ethyl acetate extract obtained after hexane and dichloromethane extraction showed SPF values of 5.30 and 4.16, respectively. Thus, the tested preparations of *P. thyrsoiflorus* demonstrated moderate variation in SPF activity, with the post-fractionation methanol extract producing the highest value among the flower preparations (**Figure 3.1**).

Overall, the chloroform and ethyl acetate fractions of *S. haematodes* root exhibited the highest SPF values among all samples investigated in the present study. The relatively high SPF values observed for these two fractions may be associated with the presence of UV-absorbing phytoconstituents concentrated during solvent fractionation. Differences in SPF among the fractions may reflect differences in the polarity and chemical composition of the compounds extracted by the respective solvents. However, the specific constituents responsible for the observed SPF activity cannot be established from the present spectrophotometric analysis alone and require further phytochemical investigation.

The UV absorption spectra of the tested samples further demonstrated their ability to absorb radiation within the UV region. The comparatively prominent absorption observed for the chloroform and ethyl acetate fractions of *S. haematodes* root is consistent with their higher SPF values (**Figure 3.2**).

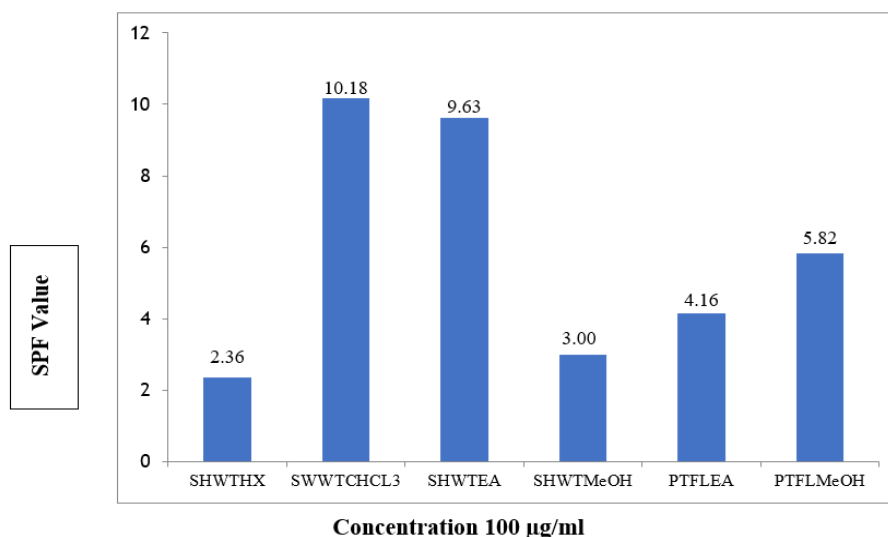


Figure 3.1: Sunscreen Protection Factor values of various fractions of *S. haematodes* root and *P. thyrsiflorus* flower fractions. Data are expressed as mean $\pm$ standard deviation ($n=3$). (SHWTHX $\rightarrow$ n-hexane extract of *S. haematodes*; SHWTCHCL3 $\rightarrow$ chloroform extract of *S. haematodes*; SHWTEA $\rightarrow$ ethyl acetate extract of *S. haematodes*; SHWTMeOH $\rightarrow$ methanol extract of *S. haematodes*; PTFLEA $\rightarrow$ ethyl acetate extract of *P. thyrsiflorus*; PTFLMeOH $\rightarrow$ methanol extract of *P. thyrsiflorus*).

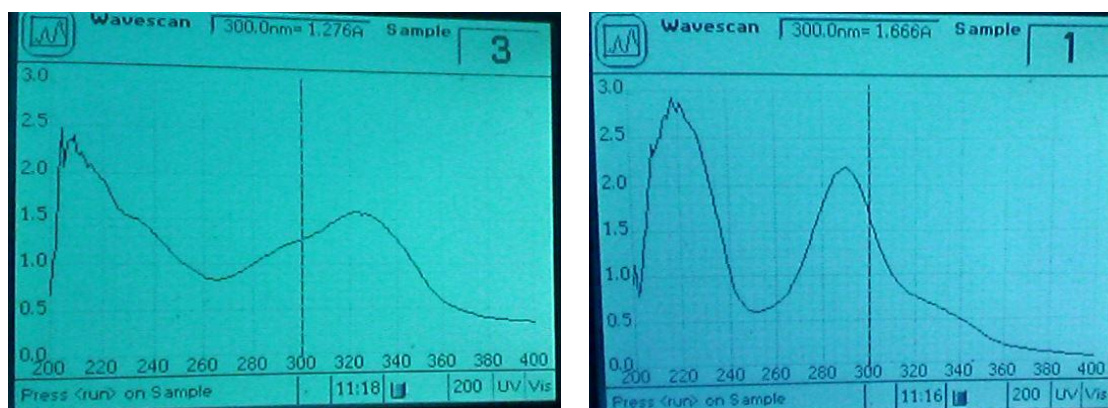


Figure 3.2: UV Absorption spectra of chloroform and ethyl acetate fractions of *S. haematodes* root solvent fractions at 100 $\mu\text{g/ml}$ of Wavelength (nm) vs Abs.

SPF Values of *Phlogacanthus thyrsiflorus* Flower Extracts

Table 1 presents the absorbance values recorded at 290-320 nm and the corresponding SPF calculations for the different *P. thyrsiflorus* flower preparations. The absorbance values varied across the investigated wavelengths and among the extracts. The calculated SPF values were 5.83, 5.30 and 4.16 for the methanol extract obtained after hexane, dichloromethane and ethyl acetate extraction, direct methanol extract, and ethyl acetate extract obtained after hexane and dichloromethane extraction, respectively.

Table 1: *In-vitro* SPF values of different extracts of *Phlogacanthus thyriflorus* flower at 100 µg/mL.

Wavelength (nm)	MeOH extract after hexane, DCM and EtOAc extraction	Direct MeOH extract	EtOAc extract after hexane and DCM extraction	EE × I
290	0.883	0.813	0.794	0.0510
295	0.824	0.758	0.733	0.0817
300	0.789	0.725	0.657	0.2874
305	0.765	0.703	0.558	0.3278
310	0.795	0.725	0.531	0.1864
315	0.766	0.712	0.415	0.0839
320	0.889	0.843	0.473	0.0180
SPF	5.83	5.30	4.16	—

Values represent measurements obtained at 100 µg/mL. DCM = dichloromethane; EtOAc = ethyl acetate; MeOH = methanol.

SPF Values of *Salvia haematodes* Root Fractions

The SPF values of the different extracts and solvent fractions of *S. haematodes* root are presented in **Table 2**. The chloroform fraction showed the highest SPF value (10.18), followed closely by the ethyl acetate fraction (9.63). The remaining preparations showed lower SPF values ranging from 2.36 to 4.13. The higher SPF values observed in the chloroform and ethyl acetate fractions suggest that these fractions contained a greater proportion of compounds capable of absorbing UVB radiation under the experimental conditions. In contrast, the relatively lower SPF values of the n-hexane and other fractions indicate comparatively weaker UVB-absorbing activity.

Table 2: *In-vitro* SPF values of different extracts and solvent fractions of *Salvia haematodes* root at 100 µg/mL.

Wave length (nm)	MeOH extract after hexane, DCM and EtOAc extraction.	n-hexane part of EtOAc extract	Chloroform part of EtOAc extract	EtOAc part of EtOAc extract	n-butanol part of EtOAc extract	EtOAc extract after hexane and DCM extraction.	EE X I	MeOH extract after hexane, DCM and EtOAc extraction.	n-hexane part of EtOAc extract	Chloroform part of EtOAc extract	EtOAc part of EtOAc extract	n-butanol part of EtOAc extract	EtOAc extract after hexane and DCM extraction.
290	0.677	0.315	0.968	2.266	0.636	0.685	0.015	0.01	0.004	0.015	0.034	0.009	0.0103
295	0.586	0.345	1.106	2.08	0.603	0.687	0.0817	0.048	0.028	0.09	0.169	0.049	0.056
300	0.496	0.333	1.281	1.704	0.53	0.627	0.029	0.014	0.009	0.037	0.049	0.015	0.018
305	0.4	0.302	1.351	1.293	0.447	0.547	0.328	0.131	0.099	0.443	0.424	0.147	0.179
310	0.353	0.322	1.461	1.052	0.408	0.522	0.187	0.066	0.06	0.272	0.196	0.076	0.097
315	0.304	0.336	1.571	0.901	0.376	0.512	0.083	0.025	0.028	0.132	0.076	0.032	0.0429
320	0.268	0.341	1.635	0.825	0.348	0.515	0.018	0.005	0.006	0.029	0.014	0.006	0.009
								0.299	0.236	1.018	0.963	0.334	0.413
SPF								3	2.36	10.18	9.63	3.34	4.13

Values represent measurements obtained at 100 µg/mL. DCM = dichloromethane; EtOAc = ethyl acetate; MeOH = methanol.

Comparative Photoprotective Potential

Comparison of the SPF values demonstrated that the *S. haematodes* root fractions generally exhibited greater SPF activity than the tested *P. thyriflorus* flower

preparations. The highest SPF was observed for the chloroform fraction of *S. haematodes* (10.18 ± 0.12), followed by its ethyl acetate fraction (9.63 ± 0.08). The highest SPF recorded for *P. thysiflorus* was 5.83.

The differences among the solvent fractions suggest that the distribution of UV-absorbing constituents may vary considerably according to solvent polarity. The higher activity of the chloroform and ethyl acetate fractions of *S. haematodes* may indicate enrichment of compounds with suitable chromophoric groups capable of absorbing UV radiation (Núñez-Pons, L., et al., 2018; Singh, V. K., et al., 2023). However, this interpretation remains preliminary because the present study did not chemically identify the compounds responsible for the observed activity.

The findings are consistent with previous reports demonstrating that plant extracts and fractions can exhibit measurable *in-vitro* SPF activity when assessed using UV-visible spectrophotometric methods (Mishra et al., 2012; Santhanam et al., 2013). Nevertheless, spectrophotometric SPF values should be interpreted as an initial indication of photoprotective potential rather than direct evidence of clinical sunscreen efficacy. Further investigation involving phytochemical characterization, concentration-dependent SPF assessment, formulation studies, photostability testing and appropriate *in-vivo* or clinical evaluation would be necessary before practical sunscreen applications can be established.

Overall Finding

The present investigation identified appreciable *in-vitro* SPF activity in several extracts and solvent fractions of both medicinal plants. Among all samples tested, the chloroform and ethyl acetate fractions of *S. haematodes* root demonstrated the most prominent SPF activity, with values of 10.18 ± 0.12 and 9.63 ± 0.08 , respectively. These findings indicate that *S. haematodes* root, particularly its chloroform and ethyl acetate fractions, warrants further investigation as a potential botanical source of photoprotective constituents.

CONCLUSION

The present study demonstrated that extracts and solvent fractions of *S. haematodes* root and *P. thysiflorus* flower possess varying degrees of *in-vitro* sun protection factor activity. Among the samples evaluated, the chloroform and ethyl acetate fractions of *S. haematodes* root showed the highest SPF values, 10.18 ± 0.12 and 9.63 ± 0.08 , respectively, while the tested *P. thysiflorus* flower preparations exhibited comparatively lower SPF values. These findings indicate that *S. haematodes* root, particularly its chloroform and ethyl acetate fractions, may contain UV-absorbing constituents with promising photoprotective potential. However, the present findings are based on an *in-vitro* spectrophotometric SPF assessment and therefore should not be interpreted as evidence of clinical sunscreen efficacy. Further studies involving phytochemical characterization, concentration-dependent SPF evaluation, photostability, formulation development, and appropriate *in-vivo* or clinical investigations are necessary to establish the practical potential of these plant fractions as natural photoprotective agents.

Consent for Publication

Not applicable.

Competing Interests

Authors have declared that no competing interests exist.

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