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Spectrophotometric evaluation of sun protection and antioxidant potential of *Artemisia maritima* L. and *Sophora mollis* Royle from Hunza, Gilgit-Baltistan

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Abstract: **Background**: Excessive ultraviolet (UV) radiation in mountainous regions increases the risk of skin disorders, highlighting the need for effective natural photoprotective agents. **Objectives**: This study aims to evaluate the sun protection factor (SPF) and antioxidant potential of two underexplored plants, *Artemisia maritima* and *Sophora mollis*, collected from high-altitude areas of Gilgit-Baltistan, to assess their suitability as natural photoprotective agents. **Methods**: UV-Vis spectroscopy was used to assess free radical scavenging activity (DPPH and ABTS assays), along with total phenolic content (TPC) and total flavonoid content (TFC). **Results**: *A. maritima* exhibited a higher SPF value (17.27 ± 0.31) compared to *S. mollis* (7.68 ± 0.18). Similarly, *A. maritima* showed greater TPC (345.93 ± 0.62 mg GAE/g) and TFC (239.30 ± 0.47 mg QE/g) than *S. mollis* (297.38 ± 0.34 mg GAE/g and 55.26 ± 0.75 mg QE/g, respectively). Antioxidant activity, measured by IC_{50} values, was strongest for Trolox (50.45 ± 0.97 µg/mL), followed by *A. maritima* (119.52 ± 2.23 µg/mL) and *S. mollis* (244.46 ± 8.06 µg/mL). In ABTS assays, *A. maritima* and *S. mollis* inhibited 68.3% and 60.2% of free radicals, respectively. **Conclusion**: These findings suggest that *A. maritima* possesses strong photoprotective and antioxidant properties, highlighting its potential as a promising natural sunscreen candidate, while *S. mollis* may provide moderate photoprotection suitable for complementary cosmetic or dermatological formulations.

Keywords: *Artemisia maritima* L; Antioxidants; Gilgit Baltistan; Hunza; Photochemistry; *Sophora mollis* Royle; Sun protection factor (SPF)

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INTRODUCTION

Hunza, located in Gilgit-Baltistan at 36.3167°N and 74.6333°E, is renowned for its rich and diverse flora. Many plant species from this region remain scientifically underexplored, despite their significant botanical and medicinal value (Hussain, 2024). Due to its high-altitude environment, Gilgit-Baltistan is exposed to extreme climatic conditions. As a result, elevated ultraviolet (UV) radiation often causes skin erythema and other disorders, which may progress to skin cancer (Ali *et al.*, 2023). To counteract these harmful effects, various skincare products such as sunscreens, moisturizers and lotions have been developed to protect against UV-A and UV-B rays. However, concerns remain about the potential health risks associated with synthetic chemicals in such formulations. This has shifted research focus toward natural alternatives, particularly the use of plant-derived compounds in sunscreens, with the intention to develop safer and more sustainable skincare products (He *et al.*, 2021). Traditional medicine in Hunza reflects this practice, as local physicians (Hakeem) use shrubs with bioactive compounds for therapeutic purposes. *Artemisia maritima* (family Asteraceae), locally known as "Rooner" or "Zoon" (Fig. 1a), has been used in folk remedies as a tincture for fever, skin infections and jaundice and as an ointment for joint pain (Awuchi & Morya, 2024). Members of this family are also known for their anticancer, antimalarial, antibacterial and anti-inflammatory activities (Ibrahimova *et al.*, 2023). Similarly, *Sophora mollis* Royle (family Fabaceae) (Fig. 1b) has long been applied in traditional medicine, where its shoots and branches are used for skin ailments (Anjum *et al.*, 2019), floral tips for digestive disorders and stems for treating jaundice, diarrhea and urinary infections (Quradha *et al.*, 2021). The chemicals derived from these shrubs easily respond to oxidative stress, a condition linked to frequent human diseases (Dumanović *et al.*, 2021). Phytochemical studies reveal that *A. maritima* contains artemisinin, coumarins, phenols, flavonoids, essential oils, lactones and traces of alkaloids, while *S. mollis* is rich in alkaloids, flavonoids, phenols and essential oils (Abate *et al.*, 2022). Of particular importance, flavonoids and phenols are recognized as potent antioxidants due to their ability to scavenge free radicals and mitigate oxidative stress (Mir *et al.*, 2024; Yamauchi *et al.*, 2024). Since oxidative stress is linked to numerous chronic diseases, identifying plants with strong antioxidant activity is vital for developing protective agents against free radical-induced cellular damage.

Recent studies have provided insights into the pharmacognostic, phytochemical and biological properties of *Artemisia maritima* and *Sophora mollis*. For

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instance, a pharmacognostic evaluation of *A. maritima* highlighted its diverse chemical and biological characteristics, contributing to its potential as a medicinal plant. Similarly, research on *S. mollis* has identified various bioactive compounds and biological activities, highlighting its therapeutic potential. (Bisht *et al.*, 2021; Trendafilova *et al.*, 2021; Ekiert *et al.*, 2022; Minda *et al.*, 2022; Nurlybekova *et al.*, 2022; Bordean *et al.*, 2023). Despite these advancements, there remains a gap in understanding their UV-protective and antioxidant activities. This study aims to address this gap by evaluating the sun protection factor (SPF) and antioxidant potential of these plants, thereby contributing to the development of natural alternatives for skin erythema and other skin conditions.

Fig. 1: a) *Artemisia maritima* L. and b) *Sophora mollis* Royle in natural habitat

MATERIALS AND METHODS

Chemicals

Methanol, Distilled water, F.C reagent (Folin-Ciocalteu), Sodium carbonate ($NaCO_3$), Aluminum chloride ($AlCl_3$10%), Sodium Hydroxide (NaOH 1M), DPPH solution, Sodium nitrite ($NaNO_2$ 5%), ABTS solution, Potassium per sulfate ($K_2H_2O_8$), Potassium hydrogen phosphate (K_2HPO_4), Phosphate Buffer Saline (PBS) and Sodium chloride (NaCl)

Sample preparation and extraction

Fresh samples of *Artemisia maritima* L. and *Sophora mollis* Royle were collected from Khanaabad village, Lower Hunza, Gilgit-Baltistan, Pakistan. The specimens were identified by Prof. Dr. Zaheer Ud Din Khan, Department of Botany, Government College University Lahore, as *Artemisia maritima* L. (herbarium number GC. Herb. Bot. 3017) and *Sophora mollis* Royle (herbarium number GC. Herb. Bot. 2793).

The fresh plant samples were air-dried under ambient conditions for 20 days. Afterward, they were ground into fine powder using a mechanical grinder. Methanol was chosen as the extraction solvent due to its ability to efficiently extract various bioactive compounds, particularly phenolic and flavonoid content. For extraction, 2.5 grams of the dried powdered sample from each plant species were placed in separate 100 mL beakers. Each beaker was filled with 25 mL of methanol and the samples were heated over a flame for a short duration and then allowed to cool. The cooled samples were kept at room temperature for 48 hours, with occasional shaking to promote extraction and increase yield. Filtration was performed using Whatman No. 3 filter paper to remove any residual impurities. The resulting filtrates were collected into separate conical flasks. Observations showed that *A. maritima* produced a more intense coloration and higher yield compared to *S. mollis*, likely due to the greater abundance of bioactive compounds in the *Artemisia* species.

SPF determination

In vitro SPF was assessed using a UV-vis spectrophotometer. Working solutions of *A. maritima* and *S. mollis* crude methanolic extracts were prepared at concentrations of 100, 300, 500, 700 and 900 µg/mL. The absorbance of these solutions was measured in the UV-B region of the spectrum (290–320 nm) at 5 nm intervals, following the methodology outlined in previous studies (Li *et al.*, 2023). Finally, via the absorbance values obtained, the SPF of the extracts was measured, taking the Mansur equation (Reis Mansur *et al.*, 2016), given below.

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

Here, the correction factor (CF) was applied, based on a recognized SPF standard. Measurements made to estimate the solar spectral irradiance (I)λ, the absorbance at a provided wavelength (abs) λ and the erythemal effectiveness (EE) λ. The experimentation for each concentration was performed thrice to obtain the standard deviations.

Total phenolic content (TPC) determination

To quantify the phenolic content, a calorimetric assay based on the Folin-Ciocalteu reagent was used (Lawag *et al.*, 2023). Diluted solutions (100–500 µg/mL) were prepared from the stock methanolic extract of *A. maritima* and *S. mollis*. For each test, 40µL of the extract was added to a test tube, followed by 20µL of Folin-Ciocalteu reagent and 3.16 mL of distilled water. The solution was incubated for 8 minutes before adding 600µL of 7.5% Na_2CO_3 solution. The reaction mixtures were incubated for an additional 30 minutes at room temperature, during which a color change occurred. The absorbance of the samples was measured at 765 nm using a UV-vis spectrophotometer. A calibration curve was generated using gallic acid as a reference standard and the phenolic content was expressed as milligrams of gallic acid equivalent per gram of extract (GAE/g) (Salim *et al.*, 2024).

Total flavonoid content (TFC) determination

The aluminum chloride calorimetric method is a well-established technique for quantifying flavonoids (Keng *et al.*, 2024). This method was applied to determine the

flavonoid content of *A. maritima* and *S. mollis*. Working solutions of both plant extracts, ranging from 100 to 500 µg/mL, were prepared by diluting the stock solution. For the analysis, 250 µL of the diluted methanolic extract from each plant (in powdered form) was transferred into separate test tubes. To each test tube, 1.25 mL of distilled water and 75 µL of 5% $NaNO_2$ were added. The mixture was incubated for 5 minutes, after which 150 µL of $AlCl_3$ solution was introduced. After another 6 minutes of incubation, 500 µL of 1M NaOH solution and 275 µL of distilled water were added. The absorbance of the final reaction mixture was measured at 510 nm using UV-Vis spectrophotometry. The TFC was calculated and expressed as quercetin equivalents per gram of extract (QE/g) (Shraim *et al.*, 2021).

DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant assay

The DPPH assay is widely used to evaluate the free radical scavenging capacity of antioxidants, which is essential for combating oxidative stress (Gulcin & Alwasel, 2023). To perform the assay, a 0.1 mM DPPH solution was prepared by dissolving 3.94 mg of DPPH in 100 mL of methanol. 250 µL aliquots of the plant extract solutions (concentrations ranging from 100 to 500 µg/mL) were added to cuvettes containing 2.5 mL of the DPPH methanolic solution. The mixtures were shaken thoroughly and allowed to react at room temperature for 30 minutes. The absorbance of the resulting solution was then measured at 515 nm, using methanol as a blank control. The percentage of DPPH scavenging was calculated using the following formula:

$$\text{Scavenging effect (\%)} = \frac{[Ao\ (Absorbance\ of\ control) - As\ (Absorbance\ of\ sample)]}{Ao\ (Absorbance\ of\ control)} \times 100$$

Where A_o is the absorbance of the control and A_o is the absorbance of the sample. The results were compared to a calibration curve constructed with Trolox, a standard antioxidant. Three independent measurements were performed for accuracy.

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay

The ABTS assay, also known as the decolorization assay, is an effective method for assessing antioxidant potential by measuring the ability to neutralize free radicals. This method was adapted from previously published protocols (Cano *et al.*, 2023). To generate the $ABTS^{\bullet+}$ cation radical, a 7 mM ABTS solution was reacted with 2.5 mM $K_2S_2O_8$ and incubated in the dark for 15 hours at room temperature. After the incubation period, the $ABTS^{\bullet+}$ solution was diluted with either methanol or phosphate-buffered saline (PBS) to achieve an absorbance of 0.706 at 734 nm. For the assay, 10 µL of the methanolic plant extract (at varying concentrations) was added to cuvettes containing 2.99 mL of the diluted $ABTS^{\bullet+}$ solution. The mixtures were intermittently shaken and allowed to react for 30 minutes. The absorbance was recorded at 734 nm and the antioxidant activity was expressed as the percentage of $ABTS^{\bullet+}$ inhibition or scavenging, using the same formula as the DPPH assay (Wołosiak *et al.*, 2022).

$$\text{Scavenging effect (\%)} = \frac{[Ao\ (Absorbance\ of\ control) - As\ (Absorbance\ of\ sample)]}{Ao\ (Absorbance\ of\ control)} \times 100$$

Statistical analysis

In this research, each test was done three times. The means ± SE (n = 3) express the statistics for each experiment. Pearson's correlation coefficient examined the relationship between various antioxidant components and antioxidant assays.

RESULTS

In this study, the SPF values for *A. maritima* and *S. mollis* methanolic extracts were measured at various concentrations ranging from 100 to 900 µg/mL. The SPF values for *A. maritima* ranged from 2.49 ± 0.31 to 17.27 ± 0.31, while *S. mollis* showed values from 2.58 ± 0.12 to 7.68 ± 0.12 (Table 1). These SPF values are relatively low compared to other *Artemisia* species, suggesting that the extracts may be more effective when combined with other sunscreen ingredients.

For the antioxidant analysis, a concentration series from 100 to 500 µg/mL was prepared for both plant species. Spectrophotometric testing revealed that both species exhibit substantial antioxidant potential, with higher concentrations correlating to increased phenolic and flavonoid content. At specific concentrations, *A. maritima* exhibited higher Total Phenolic Content (TPC: 345.93 ± 0.62 mg GAE/g) and Total Flavonoid Content (TFC: 239.30 ± 0.47 mg QE/g) compared to *S. mollis* (TPC: 297.38 ± 0.34 mg GAE/g; TFC: 55.26 ± 0.76 mg QE/g) (Fig. 2). This indicates that the presence of bioactive compounds directly correlates with antioxidant activity.

The correlation between SPF and both TPC and TFC was further analyzed using regression analysis (Fig. 3). For *A. maritima*, a trend was observed between SPF and phenolic content ($r = 0.64$, $p = 0.06$), indicating a potential relationship, though it did not reach statistical significance (Fig. 3a). Similarly, *S. mollis* showed a weaker correlation between SPF and phenolic content ($r = 0.56$, $p = 0.08$) (Fig. 3b), also lacking statistical significance. However, no clear correlation was found between SPF and flavonoid content in either species: *A. maritima* ($r = 0.12$, $p = 0.55$) (Fig. 3c) and *S. mollis* ($r = 0.34$, $p = 0.25$) (Fig. 3d). These results suggest that while phenolic content appears to have a potential influence on SPF, the correlation is not statistically conclusive and flavonoid content does not significantly impact SPF values in either species.

The antioxidant potential of the plant extracts was also evaluated using the DPPH and ABTS assays. For the DPPH assay, the plant extracts and the standard Trolox were tested over a range of concentrations (100 to 500 µg/mL). At 500 µg/mL, Trolox showed 98% inhibition,

while *A. maritima* and *S. mollis* exhibited 81% and 73.5% inhibition, respectively (Fig. 4a). These results demonstrate that both plants exhibit significant antioxidant activity, although *A. maritima* shows slightly higher scavenging potential. The half-maximal inhibitory concentration (IC_{50}) values were calculated for the plant extracts and Trolox. *A. maritima* had an IC_{50} of 119.52 ± 2.24 µg/mL, while *S. mollis* showed a higher IC_{50} of 244.46 ± 8.06 µg/mL (Fig. 4b).

Trolox exhibited a lower IC_{50} of 50.45 ± 0.97 µg/mL, confirming its stronger antioxidant activity. However, both plant extracts demonstrated noteworthy antioxidant properties, with *A. maritima* showing substantially higher activity than *S. mollis*. Further analysis of the ABTS assay showed that at 500 µg/mL, both plant extracts exhibited strong ABTS radical scavenging activity. *A. maritima* inhibited 68.3% of the ABTS radical, while *S. mollis* showed 60.2% inhibition. The Trolox Equivalent Antioxidant Capacity (TEAC) values were calculated for each extract, with *A. maritima* showing TEAC values ranging from 4.3 to 7.1 mM/g and *S. mollis* showing values between 3.8 and 7 mM/g. The highest TEAC value was observed for *A. maritima* (Fig. 5), demonstrating a positive correlation between antioxidant capacity and TFC. For *A. maritima*, the correlation was strong (R^2 = 0.9866, R = 0.9932), while for *S. mollis*, the correlation was moderate (R^2 = 0.8599, R = 0.9273).

DISCUSSION

Both *Artemisia maritima* and *Sophora mollis* contain distinct chemical and biological properties that contribute to their antioxidant activities, which play a crucial role in mitigating oxidative stress, a key factor in skin erythema and other UV-induced skin conditions (Joshi *et al.*, 2022). Interest in plant-based remedies has grown due to their potential advantages: they are often more cost-effective, safer and less toxic compared to synthetic compounds (Chrysargyris *et al.*, 2020). Therefore, the plant species were used in this study to specifically assess their effectiveness in protecting the skin from UV radiation and subsequent oxidative damage. Flavonoids, which are abundant in *A. maritima* and *S. mollis*, have been recognized as potent natural sunscreen agents due to their ability to absorb UV radiation, especially in the 290-400 nm range, crucial for protecting the skin damage (Chen *et al.*, 2022). While the SPF values observed in this study for both plants were lower than those reported for other Artemisia species, they still exhibited significant UV-absorbing properties, with *A. maritima* showing a relatively higher SPF. These findings suggest that, although effective on their own, these plant extracts may offer more potent UV protection when incorporated into a broader sunscreen formulation. Furthermore, the antioxidant potential of these extracts plays a vital role in preventing oxidative stress, which is a key factor in skin aging, erythema and even skin cancer.

Our results align with studies showing that the phenolic content of plant extracts significantly contributes to their SPF values (Mewada & Shah, 2023). Plants high in flavonoids and phenolic compounds are also effective in scavenging reactive oxygen species (ROS), which are generated by UV exposure and contribute to skin damage (Speisky *et al.*, 2022). In this study, we observed that *A. maritima* had higher levels of total phenolic and flavonoid content compared to *S. mollis* and this correlates with the greater antioxidant potential of *A. maritima*. As noted in previous research, a higher concentration of these bioactive compounds often leads to enhanced antioxidant properties (Stanojević *et al.*, 2009). The antioxidant properties of a plant are primarily assessed by quantifying TPC and TFC. The results for TFP and TPC can be further compared with other studies that found Artemisia species contain significant content of these bioactive compounds that enhance their antioxidant potential (Trifan *et al.*, 2022).

In this study, the correlation between SPF and the total phenolic and flavonoid content was analyzed using regression analysis. The results indicated a strong correlation between SPF and phenolic content, suggesting that phenols play a significant role in providing UV protection. On the other hand, the correlation between flavonoids and SPF was weaker, indicating that flavonoids, although contributing to antioxidant activity, may not be as directly involved in UV protection as phenolic compounds. This finding is consistent with previous studies on *Artemisia* species, which highlighted a strong relationship between SPF values and phenolic content (Stanciauskaite *et al.*, 2022). Our results further support the idea that increasing the concentration of bioactive compounds, particularly phenols, in plant extracts leads to an increase in their SPF values, providing more effective protection against UV radiation. This observation is consistent with the FDA's recommendation that sunscreen formulations should have an SPF greater than 15 to provide effective protection (Kandemir, 2023).

The DPPH assay conducted in this study demonstrated significant antioxidant activity for both plant extracts. Although Trolox, the standard antioxidant, exhibited superior activity, *A. maritima* and *S. mollis* also showed strong free radical scavenging potential, suggesting their utility in mitigating oxidative stress caused by UV exposure. Notably, the antioxidant potential of *S. mollis* has not been extensively studied, but this study provides strong evidence of its considerable antioxidant capacity, which could be beneficial for preventing skin erythema and other UV-induced skin damage. The ABTS assay further confirmed the antioxidant properties of both plants, where the antioxidant capacity was quantified by observing the decolorization of the $ABTS^{\bullet+}$ radical solution. As antioxidants neutralize the free radicals, the color intensity of the solution decreases, enabling accurate TEAC value calculations (Christodoulou *et al.*, 2022).

Table 1: SPF values of methanolic extracts of *Artemisia maritima* L. and *Sophora mollis* Royle at different concentrations between 290 to 320nm UV absorption with a 5nm difference using the Mansur equation

(nm)	EE*I (Normailzed)	*Artemisia maritima L.*					*Sophora mollis royale*				
		100 ug\ml	300 ug\ml	500 ug\ml	700 ug\ml	900 ug\ml	100 ug\ml	300 ug\ml	500 ug\ml	700 ug\ml	900 ug\ml
290	0.0150	0.429	0.746	1.104	1.432	1.821	0.327	0.456	0.666	0.850	1.077
295	0.0817	0.393	0.700	1.053	1.367	1.802	0.300	0.429	0.642	0.829	1.055
300	0.2874	0.367	0.679	1.004	1.311	1.791	0.276	0.404	0.615	0.800	1.029
305	0.3278	0.340	0.651	0.953	1.255	1.743	0.255	0.378	0.585	0.766	0.990
310	0.1864	0.326	0.460	0.946	1.210	1.637	0.235	0.356	0.580	0.733	0.960
315	0.0837	0.324	0.653	0.988	1.289	1.603	0.221	0.336	0.529	0.696	0.916
320	0.0180	0.321	0.682	1.019	1.372	1.541	0.210	0.317	0.499	0.657	0.865
SPF	1	2.49± 0.31	6.30± 0.31	6.30± 0.31	12.79± 0.31	17.27± 0.31	2.58± 0.12	3.82± 0.12	5.88± 0.12	5.88± 0.12	7.68± 0.12

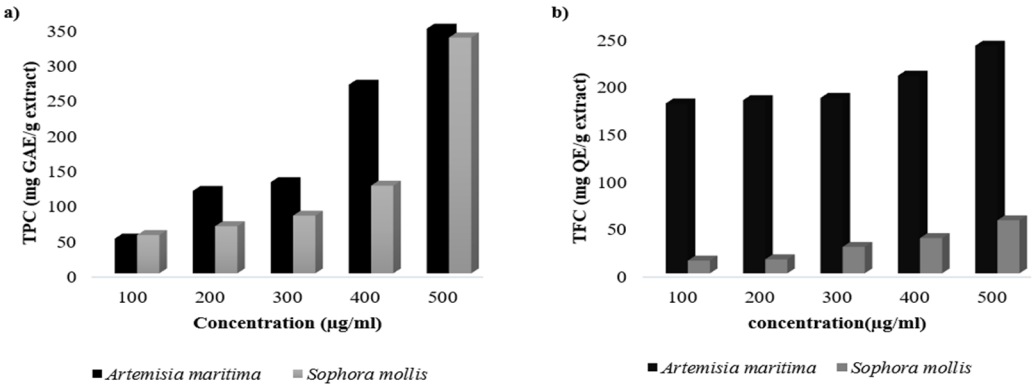

Fig. 2: a) Total phenolic content (TPC) calculated through the Folin-Ciocalteu method and b) Total flavonoid content (TFC) determined via Aluminum chloride calorimetric assay for both *A. maritima* and *S. mollis*.

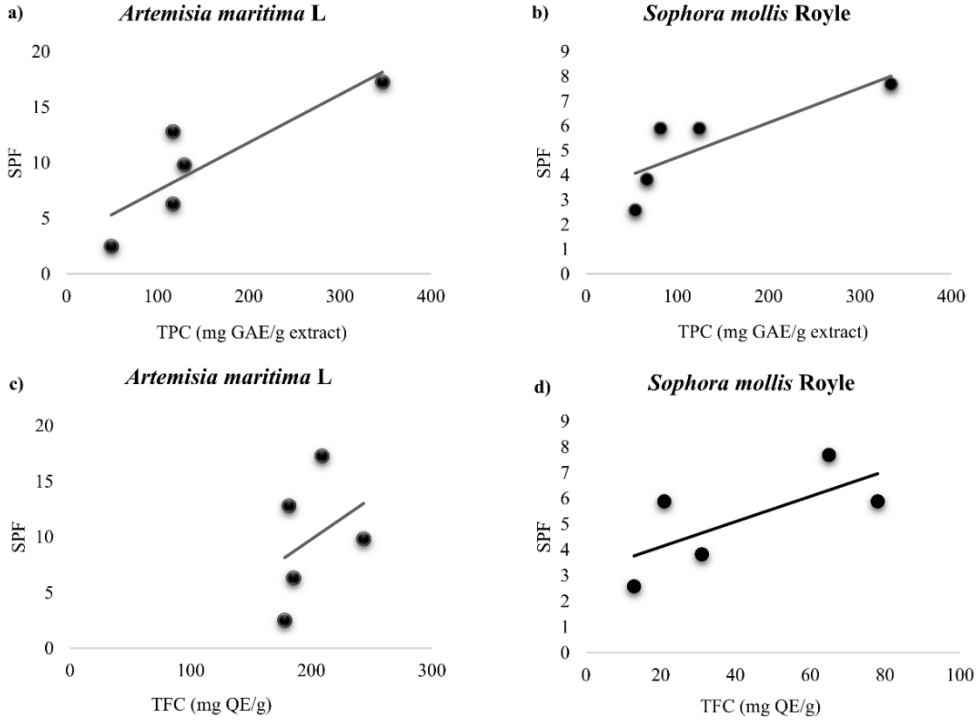

Fig. 3: Correlation between (a) SPF and TPC for *A. maritima*, (b) SPF and TPC for *S. mollis*, (c) SPF and TFC for *A. maritima* and (d) SPF and TFC for *S. mollis.*

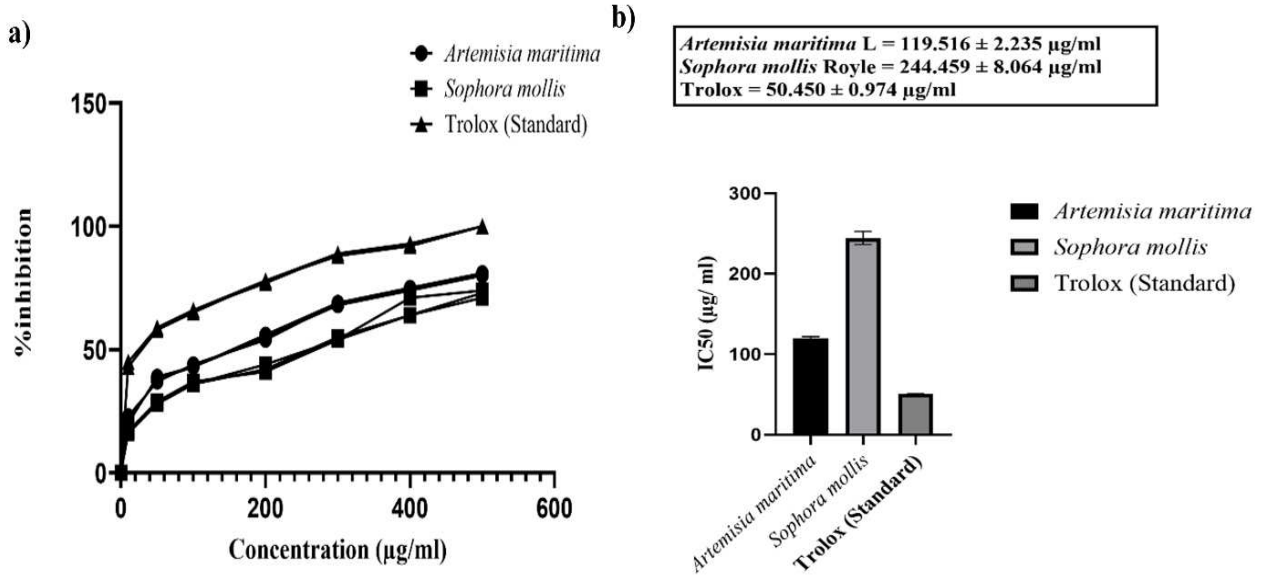

Fig. 4: (a) % DPPH scavenging of *A. maritima* and *S. mollis* extracts and Trolox standard (Mean ± SD) and (b) IC_{50} values indicating the inhibitory activity of *A. maritima* and *S. mollis* and Trolox standard (Mean ± SD).

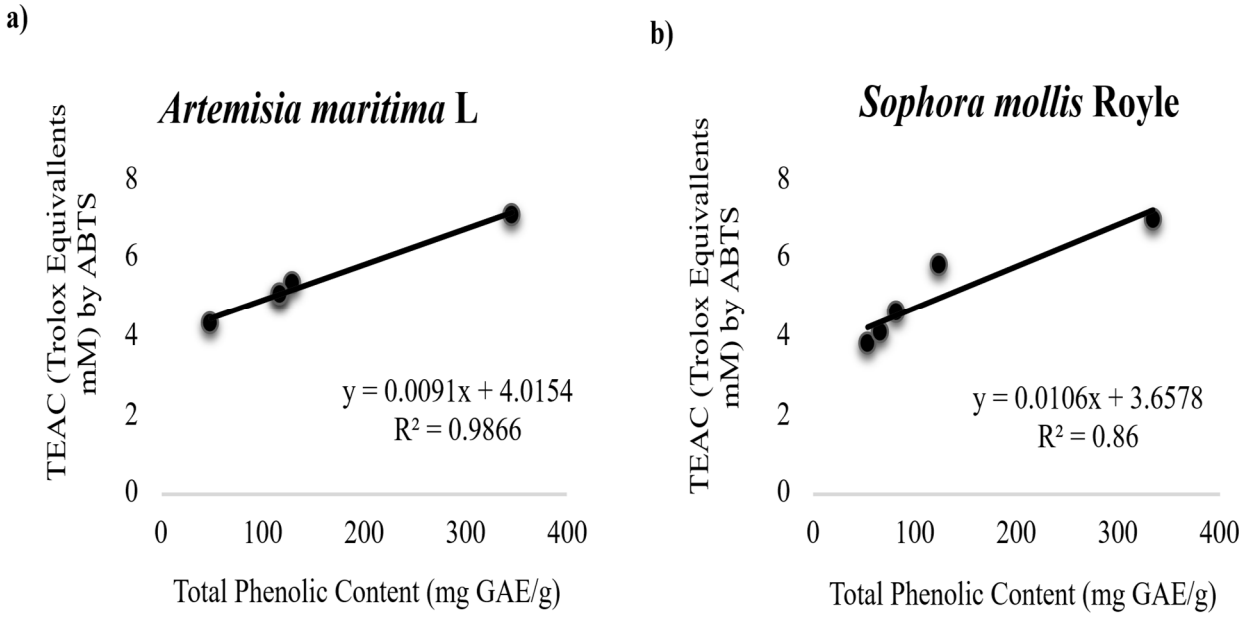

Fig. 5: Correlation between Total Phenolic Content (TPC) and Trolox Equivalent Antioxidant Capacity (TEAC) Values (mM) for *A. maritima* and *S. mollis*, showing linear relationships

The results showed that *A. maritima* exhibited the highest TEAC values, reflecting its potent antioxidant activity. TEAC values were directly correlated with total phenolic content in both plant extracts, suggesting that higher phenolic levels contribute to greater antioxidant capacity. This finding is supported by previous research, which demonstrated that high phenolic content in plants correlates with increased antioxidant effectiveness (Lu *et al.*, 2023; Salih *et al.*, 2023). The effectiveness of these plants in neutralizing free radicals, particularly DPPH and $ABTS^{\bullet+}$ radicals, further supports their potential use as natural ingredients in sunscreen and skincare products.

CONCLUSION

This study documents the significant photoprotective and antioxidant properties of *Artemisia maritima* L. and *Sophora mollis* Royle. The comparatively higher SPF value, phenolic and flavonoid content and free radical scavenging activity of *A. maritima* suggest its strong

potential as a natural source for sunscreen development. Meanwhile, *S. mollis*, though exhibiting moderate SPF and antioxidant activity, still demonstrates notable bioactive potential, making it a valuable candidate for complementary or combined formulations. These findings emphasize the importance of exploring indigenous plant resources as sustainable alternatives to synthetic sunscreen agents. Moreover, further investigation into both plants is needed to identify active components for their future use in pharmaceutical and cosmetic applications.

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Authors' contributions
Corresponding Author supervised this research, design the idea and refine the manuscript. Ms Nain Tara help in plant collection and draft the manuscript, while Ms Sundas Shahzadi along with Ms Nain Tara performed the analysis as part of their BS Chemistry research.

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Data availability statement
We can provide any relevant supplementary data on demand.

Ethical approval
As there is no human or animal studies involved therefore ethical approval is not relevant.

Conflict of interest
The authors hereby confirm that they have no competing interests, whether financial or personal, related to this study.

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