The SPF determination of Albanian mountain tea Sideritis raeseri subsp. raeseri and correlation with phenolic compounds

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Abstract. This study used the Mansur et al. method to determine the Sun Protection Factor (SPF) for the Sideritis raeseri subsp. raeseri plant (wild-growing mountain tea) and its related compounds. To achieve this, 1 gram of the plant and 1 gram of its different aerial parts (leaves, flowers, and stem) were weighed on an analytical scale and divided into separate empty beakers. By pouring 50 ml of boiled distilled water in each beaker and mixing it for 5' with 1g of dried aerial parts, water-distilled tea aqueous extracts were prepared for each group, followed by filtering and centrifuging. The optical density (O.D.) values were measured for each 20%v/v solution of plant leaves, stem, and flower (5%v/v). The mean O.D. values of three measurements (performed with a UV-VIS spectrophotometer model 756s, using distilled water as a blank reference) at wavelengths between 290 and 320 nm (with 5 nm intervals) were used to calculate the SPF in vitro using the Mansur et al. formula. Results showed that the leaf extract had the highest SPF (43.17), followed by the flower extract (13.14) and the stem extract (4.92). The SPF of the entire plant was recorded as 13.14. These findings indicate that the plant, particularly its leaves, possesses significant sun protection potential and could be suitable for use in cosmetics. The SPF values of the aerial parts were directly proportional to their phenolic content, with a strong positive correlation confirmed by Pearson's correlation coefficient ($r = 0.958$, $p < 0.01$).

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1 Introduction

In recent years, concerns have been raised about the real-world effectiveness of sunscreens with SPF, particularly in countries with extreme UV exposure. Also, research indicates a

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consistent rise in the incidence of skin cancer, including melanoma and nonmelanoma, in recent years, with the sharpest increase observed among males and older adults, largely due to inadequate sun protection [1,2,3]. There is strong evidence that sunscreen is safe to use and, when applied correctly, reduces the risk of skin cancer [4]. While UV (UVA, UVB) rays play a positive role by stimulating the production of vitamin D_3 through the irradiation of the 7-dihydro cholesterol (provitamin D_3), they also contribute to several harmful effects, such as allergic reactions, actinic lesions, photoaging, eye damage, and skin cancer [5]. On the other hand, UVC radiation, although the most biologically damaging due to its high energy, poses minimal risk to humans since the ozone layer absorbs it, rendering it biologically irrelevant. Reducing direct sun exposure between 10:30 AM and 4:00 PM to lower UV-related risks and using sun protection products, like creams or oils that absorb or block UV radiation, are highly effective preventive measures. An emerging trend in sun care involves using herbal sunscreens in the form of creams, lotions, gels, or oils, which contain natural ingredients that offer moisturizing and antioxidant benefits, or a combination of both [6]. The cosmetic industry is increasingly focusing on plants' natural defence against UV radiation. The effectiveness of sun-protective products is measured by their sun protection factor (SPF), which indicates the amount of UV energy required to cause a minimal erythema dose (MED) on protected skin versus unprotected skin.

$$\boldsymbol{SPF} = \frac{\boldsymbol{MED-protected\ skin}}{\boldsymbol{MED-unprotected\ skin}} \qquad (1)$$

Two primary methods for determining SPF are "in vivo" and "in vitro." The "in vivo" methods involve direct exposure of human skin to UV radiation, which poses certain risks, making these methods less favourable. In contrast, "in vitro" methods are safer and simpler to perform. One widely used "in vitro" technique is the Mansur method, which calculates SPF by analysing absorption characteristics of sunscreen agents through spectrophotometric measurements of their diluted solutions [7,8]. The phenolic content of tea solutions is an important factor in determining the SPF of the plants and plays a dual role in defense against UV rays.

The wild Albanian "mountain tea", Sideritis raeseri subsp raeseri (Srsrw) belongs to Lamiaceae family and was studied in this paper for its potential protective effects against direct sunlight, particularly UV radiation, and indirectly through its antioxidant properties with the "in vitro" method. This plant, typically found at elevations above 600 meters, is a mostly perennial herb that flourishes in calcareous, well-drained, slightly alkaline soils. It prefers rocky slopes with ample sunlight exposure, experiencing dry summers and mild winters. The tea, made from an aqueous solution of its aerial parts (flowers, stems, and leaves), is widely used for its anti-inflammatory, analgesic, gastroprotective, and antimicrobial benefits. Additionally, it serves as a dietary supplement for anaemia [9].

2 Materials and methods

2.1 Plant preparation

The Srsrw plant collected from Llogara Pass (Vlora) was dried at room temperature. Portions of 1 gram each from stems, leaves, flowers, and the whole plant were weighed on an analytical balance and placed into separate beakers. Each beaker received 50 mL of distilled boiling water, and the mixtures were left to steep for 5 minutes. The tea solution was filtered through a 1.2 µm filter and then squeezed to extract liquid. The filtrate was centrifuged at 2500 rpm for 5 minutes (TDZ4-WS model), and the supernatant was diluted with distilled water to a 20% v/v concentration. The diluted solutions obtained from four different types of parts (leaves, flowers, stems, and whole plants) were subjected to spectrophotometric measurements.

2.2 Spectrophotometry measurement

Absorbance measurements were taken from 290 to 320 nm at 5 nm intervals(step) using a UV-VIS spectrophotometer (NADE 756s) with a 10 mm quartz cuvette, using distilled water as a blank. Each optical density (O.D.) reading was repeated three times, and the mean absorbance values were used in the Mansur formula to calculate the SPF. Water as the solvent does not interfere with UV absorption in tea-aqueous solutions, as it primarily absorbs below 200 nm.

2.3 Mansur formula

Mansur et al. [7] developed an equation using UV spectrophotometry to calculate SPF by measuring the absorbance of diluted plant extracts (from 290 to 320 nm with a 5 nm step). This “in vitro” method offers a reliable approach to assessing the sun-protective properties of tea-aqueous solutions without requiring direct testing on humans against UVA and UVB. The SPF is calculated using the following equation (2):

$$SPF = CF\ x\ \sum_{290}^{320} EE(\lambda) x\ I(\lambda) x\ Abs(\lambda) \quad (2)$$

where *EE* is the erythemal effect spectrum, *I* is the solar intensity spectrum, $(EE(\lambda) x\ I(\lambda)$ is a constant, describing the relationship between the erythemal efficiency spectrum (EE) and the solar simulator intensity spectrum (I) at wavelengths from 290 to 320 nm[, *Abs* is the absorbance of sunscreen products, and *CF* is the correction factor (=10).

3. Results and discussions

This paper contributes to the body of research using absorbance spectrophotometry to determine the SPF "in vitro" through the mathematical formula (2) of Mansur et al. Table 1 present the absorbance measurements at seven different wavelengths and the calculated SPF values for each of the four groups.

For the leaves group, the 20% v/v solution initially measured a high O.D., causing saturation of the detector. As a result, a diluted 5% v/v solution was used instead, and the resulting O.D. values were multiplied by a dilution factor of 4 to obtain the correct readings. The corresponding SPF value calculated for this group is 42.13. Table 1 provides the O.D. values for the 5% v/v solution**.**

As shown in Table 1, the "leaves" group exhibits the highest SPF value of 43.17, followed by the "flowers" group with an SPF of 13.14, and the "stems" group with the lowest SPF of 4.92. The "plant" group has an SPF of 8.29, which is lower than the average of the individual groups' SPFs (23.48). Instead, it reflects the natural weight distribution of the plant's parts, with leaves, flowers, and stems in the ratios of 1:2.52:2.31 (17%, 43%, and 40%, respectively). On average, the dried *Srsrw* plant has 2.52 times more flowers than leaves and 2.31 times more stems than leaves, with a nearly equal proportion of flowers to stems.

Table 1. Results for SPF according to the group name

λ	EE*I	Mean O.D. leaves±SD	Mean O.D. stems+SD	Mean O.D. flowers+SD	Mean O.D. plant+SD
[nm]		(L)	(S)	(F)	(P)
290	0.015	1.164±0.001	0.538±0.000	1.375±0.000	0.859±0.001
295	0.0817	1.136±0.002	0.524±0.000	1.362±0.000	0.846±0.001
300	0.2874	1.101±0.003	0.507±0.000	1.342±0.000	0.841±0.000
305	0.3278	1.067±0.008	0.488±0.000	1.311±0.000	0.828±0.000
310	0.1864	1.051±0.016	0.473±0.001	1.281±0.000	0.812±0.000
315	0.0837	1.048±0.027	0.465±0.000	1.26±0.001	0.806±0.000
320	0.018	1.064±0.025	0.461±0.001	1.254±0.000	0.819±0.000
SPF		43.17	4.92	13.14	8.29

Mean O.D. values of group leaves are corrected by a dilution factor of 4 (5% v/v).
*EE*I & CF according to Mansur et al.[6].*
The low SD values observed for all reflect the high repeatability and the resolution limit of the spectrophotometric method, rather than true biological variability

The O.D. values are slightly higher at 290 nm than at other wavelengths (as seen in Table 1, and Fig. 1), likely due to phenolic compounds, which tend to absorb more strongly near 290 nm.

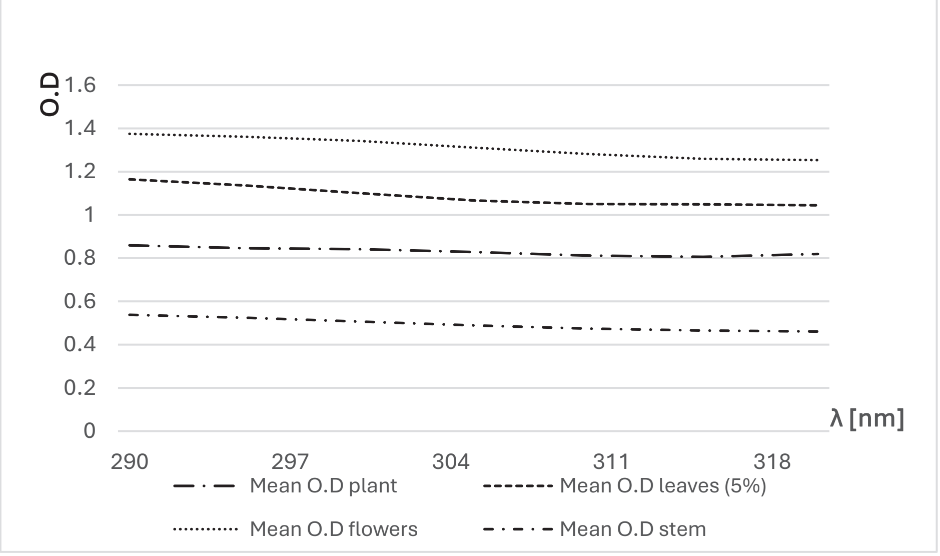

Fig. 1. Shoulder or weaker absorption tapering off for each group from 290-320nm

The absorbance spectra (Fig.2) of the tea solution of Srsrw registered an absorption peak in the UV-C range of around 278-279 nm due to the presence of Carvacrol and Thymol (Fig.3) in the tea aqueous solutions (presence of an aromatic ring with conjugated π-electrons), and weaker absorbance in the 290-320 nm range in the UV-B region. The methanol base absorption spectra of those 2 phenolic compounds register a peak at 274-276 [12,13], thus a bathochromic (red) shift has occurred due to water solution (water is more polar than methanol because it has a higher dipole moment, greater hydrogen-bonding ability, and higher dielectric constant) and slightly acidic pH. The pH values for each group were somewhat acidic, respectively: the group of "leaves" with 5.9, the group of "flowers" with 5.4, the group of "stems" with 5.8, and the group of "plants" with 5.6.

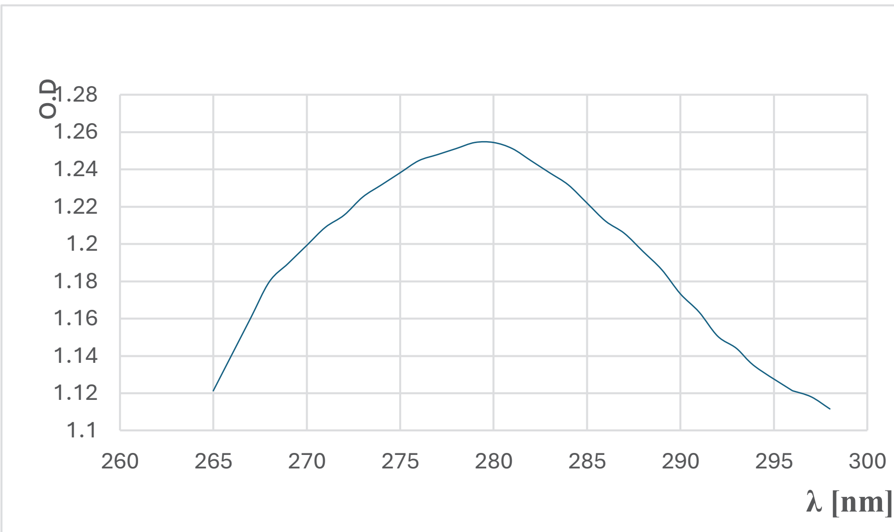

Fig. 2. Absorption spectra of 5% v/v *Srsrw* aqueous solution with a maximum at 278nm

The water solution and slightly acidic pH potentially cause a bathochromic shift in its UV absorption spectrum with a shoulder or weaker absorption tapering off from 290 nm to 320 nm.

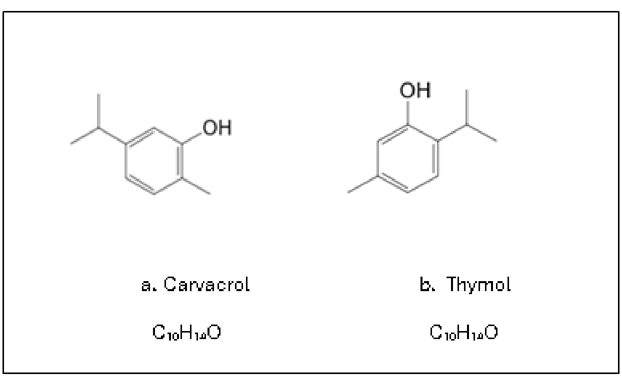

Fig. 3. Phenolic compound structure found in the tea aqueous solution of Srsrw

Various studies on Sideritis species in the Balkan Peninsula [14] have shown that the antioxidant activity (AOA) of phenolic compounds (Fig.4) in the aerial parts of this plant—when compared to Trolox as a standard—is highest in the leaves, followed by the flowers, and lowest in the stems [15].

Additional research [9, 16, 17] suggests that the antioxidant content in different *Sideritis* species across Balkan countries is quite similar. These plants demonstrate both light and direct protection potential due to their ability to absorb UV rays and their antioxidant, anti-inflammatory, and immune-modulating properties.

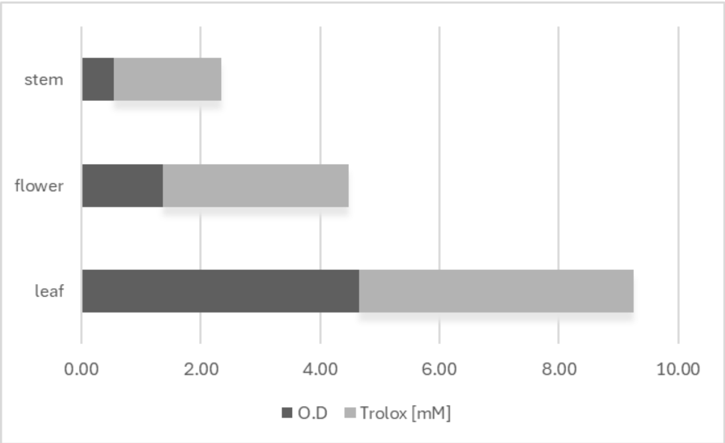

Fig. 4. Correlation between O.D. in tea-aqueous solutions and phenolic content

The correlation between O.D. values and phenolic content (based on antioxidant activity) was carried out using Pearson correlation coefficient $r=0.958$, and the p-value was significantly low ($p < 0.01$), indicating a strong positive correlation between them.

The antioxidant capacity has been positively correlated with phenolic content [10], and these compounds exert their antioxidant effects through several mechanisms. Phenolic compounds, such as carvacrol and Thymol, have been found in high percentages (respectively 36.7% and 0.5% of total essential oils) in tea-aqueous solutions of Srsr [9]. Carvacrol and Thymol both absorb UV light primarily because their chemical structure includes an aromatic ring and a functional hydroxyl group (-OH). Carvacrol and Thymol contain a benzene ring with a delocalized π-electron that absorbs energy and jumps from bonding to antibonding orbitals, π to π* transitions. A hydroxyl functional group (-OH) on the aromatic ring increases conjugation and electron density. It may also influence UV absorption by stabilizing the excited state of the molecule. Essential oils found in the water extracts of Srsrw, such as oxygenated monoterpene, oxygenated sesquiterpene, and sesquiterpene [9], do not affect absorption in the interval of 290-320nm since they do not absorb in these wavelengths. They can play an important role in scavenging free radicals and reducing oxidative stress by mitigating the damage caused by UV radiation [11].

4 Conclusions

The results show that for the same dry-weight aerial parts of wild Sideritis raeseri, the highest SPF value was found in the group “leaves” (43.17), followed by the “flowers” group (13.14), and the lowest in the group “stems” (4.92). The SPF of the entire plant was found to be 8.29. These values suggest Srsrw is a good plant for preparing sunscreen products.

The SPF values found using the Mansur method proportionally correspond with the antioxidant activity of phenolic compounds in plant aerial parts tea solutions.

Protection occurs on two levels. The first level involves the stress and absorption of UV light from the structure of phenolic molecules, and the second level consists of the scavenging of free radicals, reducing oxidative stress, and mitigating damage caused by UV radiation from phenolic compounds and certain essential oils.

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