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Interpretation of the antioxidant potential of *Salix purpurea* subsp. *leucodermis* Yalt. and *Salix caprea* L. using Python

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Abstract: This study explores the medicinal potential of two willow species, *Salix purpurea* subsp. *leucodermis* Yalt. and *Salix caprea* L., with a particular focus on their antioxidant properties. Willows are well known for their therapeutic applications, largely attributed to their phenolic and flavonoid compounds, which exhibit significant bioactive potential. In this research, we quantified the total phenolic and flavonoid contents in different plant parts—leaves, bark, and twigs—collected from distinct habitats. Considering the results of total phenolic and flavonoid content, it was observed that DPPH radical scavenging activity is notably elevated in the bark and leaves of the species, particularly in the NGBT and Kızılcahamam regions. Antioxidant capacity was also assessed using computational analyses performed with Python, with the goal of comparing the species' efficacy and exploring habitat influence on phytochemical composition. The results revealed significant variations in phenolic and flavonoid concentrations across species and habitats, underscoring the role of environmental factors in shaping metabolite accumulation. These findings contribute to the growing body of knowledge on the phytochemical applications of willows and highlight their potential as natural sources of antioxidants.

Keywords: *Salix caprea*, *Salix purpurea* subsp. *leucodermis*, Antioxidant potential, Python, Türkiye.

1 Introduction

Throughout history, willows have played a significant role in human culture, often found in gardens, fields, and along waterways, symbolizing both protection and ornamental beauty. In addition to inspiring literature and art, willows have long been valued for their medicinal properties. Ancient civilizations, such as those of Egypt, Sumer, and the Hittites, recognized their use in treating pain and inflammation. Assyrian clay tablets document the medicinal use of willow leaves, while traditional uses in basketry, fencing, and furniture date back to the earliest periods of human history (Alp et al. 2021). Today, beyond these traditional applications, willows are important in environmental remediation and phytochemistry.

Salix L., a genus with approximately 500 species worldwide (POWO 2024) is especially diverse in China (270 species), Russia, and Central Asia (120 species). In Türkiye, 27 species are naturally distributed (Acar et al. 2022), with *Salix purpurea* L. (purple willow) standing out for its bioenergy potential, given its rapid growth and salicin content (Carlson et al. 2019). This study focuses on *S. purpurea* subsp. *leucodermis* Yalt., an endemic subspecies first described by Yaltırık (1989). This subspecies, found in an isolated area on Mount Sandras (Muğla, Türkiye), has recently gained attention due to its medicinal and bioenergy potential (Acar et al. 2022; POWO 2024). In addition to *S. purpurea* subsp. *leucodermis*, *Salix caprea* L. (goat willow) is a key species in this study. *S. caprea* is widely used in biomass production, erosion control, and water quality management due to its cold-hardiness and ability to thrive in diverse environments (Acar and Gokok 2021). *S. caprea* has also been employed in Indian medicine for its astringent, antiseptic, and antipyretic properties (Ahmed et al. 2011; Akopov 1981). Ethnobotanical

studies show that both *S. caprea* and *S. purpurea* subsp. *leucodermis* have long been utilized in traditional medicine for treating conditions such as rheumatism, malaria, and inflammatory diseases (Jarić et al. 2015).

Willows are rich in bioactive compounds, including salicin, a precursor to salicylic acid (aspirin), as well as flavonoids, phenolic acids, and procyanidins, which contribute to their antioxidant, anti-inflammatory, and antimicrobial properties (Nahrstedt et al. 2007). While salicin alone may not fully account for the medicinal potency of willow bark, other phytochemicals, particularly polyphenols and flavonoids, are thought to enhance its antioxidant and anti-inflammatory effects (Tawfeek et al. 2021). Previous research on Turkish willow species, including *S. purpurea* subsp. *leucodermis* and *S. caprea*, has demonstrated promising antioxidant and anti-inflammatory properties (Akyürek and Acar 2020; Gligorić et al. 2019).

Given the medicinal potential of *S. purpurea* subsp. *leucodermis* and *S. caprea*, this study aims to investigate their antioxidant potential, building on earlier research into their therapeutic benefits (Ahmed et al. 2011; Devi and Periyanayagam 2010; Wiart 2007).

2 Materials and methods

2.1 Plant material

S. caprea L. and *S. purpurea* subsp. *leucodermis*' leaves, bark and flowers were collected in the spring and early summer from willow garden of National Botanical Garden of Türkiye (NBGT), Ankara/ Kızılcahamam and, Muğla/Köyceğiz provinces. The reference material, including herbarium specimens, was conserved in the TC Herbarium of the NBGT, while the living accessions were maintained in the Salix Gallery Forest of NBGT. Detailed information is provided in Table 1.

Table 1. Plant material used in the study, including locations, altitude, voucher numbers and accession numbers

No	Species name	Location	Voucher number in TC Herbarium	Altitude (m)	Sampling time	Accession number in NBGT (Salix Gallery Forest)
1	*S. purpurea* subsp. *leucodermis*	NBGT/Ankara	TC 534 (female)	800	April, 2022	2021-00006
2	*S. purpurea* subsp. l*eucodermis*	Gökova/Köyceğiz/Muğla	TC 535 (male)	1768	May, 2022	-
3	*S. caprea*	NBGT/Ankara	TC 599 (male)	800	April, 2022	2022-00063
4	*S. caprea*	Kızılcahamam/Ankara	TC 598 (sterile)	1510	May, 2022	-

2.2 Extract preparation

The collected samples were pulverized and sieved. Samples of 10 to 30 g of fresh leaves, flowers and bark were dried at room temperature for 2 weeks. After grinding, the samples were kept in a desiccator until the time of analysis. The extraction procedure was completed in accordance with the method described by Cai et al. (2004) for the analysis of total phenolic and flavonoid contents, as well as antioxidant activity. For the extraction process, 1 g of dry plant samples were weighed, 19 ml of 80 % methanol (MEOH) was added and homogenized with ultra turrax (11.000 rpm, 15 sec). The samples were subjected to extraction in an orbital shaker (Unimax 2010, Heidolph, Germany) at 180 rpm for 24 hours. At the end of the extraction period, the tubes were centrifuged at 5000 rpm for 10 min at 4 °C and filtered. The analysis was done in Bati Akdeniz Agricultural Research Institute Laboratory, Antalya.

2.3 Determination of total phenolic content in the plant extracts

Total phenolic content was determined by using Folin-Ciocalteu solution according to the method proposed by Spanos and Wrolstad (1990). For this purpose, 100 µl of sample extract was taken into glass tubes, 900 µl of distilled water, 5 ml of 0.2 N Folin-Ciocalteu solution and 4 ml of 7.5 % sodium bicarbonate (Na_2CO_3) solution were added respectively, the tubes were mixed in a vortex and kept at 25°C in the dark for 2 hours. At the end of this period, the total phenolic content was calculated as mg gallic acid equivalent (GAE)/100 g dry sample by using the absorbance value read at 765 nm wavelength in spectrophotometer (Shimadzu UV-Vis 160A, Japan) and the curve prepared with gallic acid. Results were expressed as grams of gallic acid equivalent (GAE) per 100 g dry weight (DW).

2.4 Determination of total flavonoid content in the plant extracts

The spectrophotometric method described by Karadeniz et al. (2005) was used for the colorimetric determination of total flavonoid content with aluminium chloride. 1 ml of sample was placed in a 10 ml glass bottle, 4 ml of distilled water and 0.3 ml of 5 % sodium nitrite ($NaNO_2$) were added and mixed. After 5 min 0.6 ml of 10 % aluminium chloride ($AlCl_3.6H_2O$) was added and after 5 min 2 ml of 1 mol/L NaOH was added and the total volume was made up to 10 ml with distilled water. After mixing the mixture well, the total amount of flavonoids was calculated as mg catechin equivalent (CE)/100 g dry sample using the absorbance value read at 510 nm wavelength on a Shimadzu UV-Vis spectrophotometer and the calibration curve prepared with catechin.

2.5 Determination of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Antioxidant activity based on DPPH radical inhibition was determined according to Cemeroğlu (2010). Firstly, 600 µl of 1 mM DPPH radical solution prepared with pure MeOH was placed in glass tubes. Then, 5 different volumes of the sample extract were added to the tubes. Pure MeOH was added to each tube for a total volume of 6 ml. The tubes were vortexed and incubated in the dark for 15 min. To be used as a witness, 600 µl DPPH and 5400 µl MeOH were added to one tube. At the end of incubation, the absorbance value of the tube contents was read at 517 nm wavelength in UV-Vis spectrophotometer and the inhibition values corresponding to each sample volume were calculated according to the following equation (Equation 1):

$$\text{Inhibition (\%)}: [(A_{DPPH} - A_{sample})/A_{DPPH}] \times 100 \quad (1)$$

A_{DPPH}: Absorbance value of DPPH solution

A_{sample}: Absorbance value of the sample extract

The % inhibition values and concentration values obtained from the samples prepared at different concentrations were graphed and the effective concentration (EC_{50}) that reduces the effect of DPPH by 50% was calculated for each sample.

2.6 Data analysis with Python

Python programming language was applied for the data cleaning and data visualization with the use of bar graphs to interpret total phenolic and flavonoid contents. Basic data cleaning operations were performed on MS Excel Spreadsheet for miss spelling, removal of duplicates and treatment to missing values followed by importing this cleaned data spreadsheet to Python Pandas library data frame (McKinney 2010). Python data frame was applied for further detailed data wrangling for miss spelling, missing values, and duplicates. Furthermore, in case of detection of an anomaly, the labels are inspected from the previously shot photographs.

3 Result and discussion

3.1 Total phenolic and flavonoid contents

The methanolic extracts of the samples showed total phenolic and flavonoid contents ranging from 0.95 to 5.94 g of GAE/100 g DW (Table 2) and 0.39 to 2.67 g of CE/100 g DW (Table 3), respectively.

3.2 Total phenolic and flavonoid contents interpreted by Python

Preliminary Python graphics of samples from two species and three different organs (bark, catkin and leaves) show that, rather than species selection, the amount of flavonoids and phenolics varies according to the kind of organ or habitat. Overall, it was revealed that the barks of *S. purpurea* subsp. *leucodermis* and *S. caprea* species had higher total flavonoid and phenolic contents than the other organs (Figure 1).

Comparing the two species, it is found that the bark of *S. purpurea* subsp. *leucodermis* and the leaves of *S. caprea* have higher total flavonoid and phenolic contents. While the overall flavonoid and phenolic contents in the flowers were not as high as those in the bark and leaves, the amounts were higher in the flower of *S. purpurea* subsp. *leucodermis* (Figure 1).

Considering the bark and leaves of *S. caprea* in terms of habitat, the total flavonoid content was found to be the highest in Kızılcahamam location and it was found to be high in NBGT, Kızılcahamam locations, respectively. Besides, the total flavonoid content in *S. purpurea* subsp. *leucodermis* bark was high and almost unaffected by habitat change. When the bark and leaves of the two species were evaluated in terms of total phenolic content, it was seen that the highest amount was obtained from *S. caprea* individuals found in NBGT (Figure 2).

Table 2. Total phenolic content of 20 samples from *S. caprea* and *S. purpurea* subsp. *leucodermis*[a]

Scientific name	Location	Parts	Total phenolic content (g GAE / 100 g DW)[b]
S. caprea	NBGT-1	Leaf	5.94
S. caprea	Kızılcahamam/Ankara-2	Leaf	5.18
S. caprea	NBGT-1	Bark	5.04
S. purpurea subsp. *Leucodermis*	NBGT-1	Bark	4.93
S. caprea	Kızılcahamam/Ankara-3	Bark	4.70
S. caprea	Kızılcahamam/Ankara-2	Bark	4.68
S. purpurea subsp. *Leucodermis*	Gökova/Köyceğiz/Muğla-2	Bark	4.59
S. purpurea subsp. *Leucodermis*	Gökova/Köyceğiz/Muğla-3	Bark	4.54
S. caprea	Kızılcahamam/Ankara-3	Leaf	4.05
S. purpurea subsp. *Leucodermis*	NBGT-1	Leaf	3.84
S. caprea	Kızılcahamam/Ankara-1	Leaf	3.20
S. purpurea subsp. *Leucodermis*	Gökova/Köyceğiz/Muğla-1	Bark	2.87
S. purpurea subsp. *Leucodermis*	Gökova/Köyceğiz/Muğla-1	Flower	2.63
S. caprea	Kızılcahamam/Ankara-1	Bark	2.44
S. purpurea subsp. *Leucodermis*	Gökova/Köyceğiz/Muğla-3	Flower	2.19
S. purpurea subsp. *Leucodermis*	Gökova/Köyceğiz/Muğla-1	Flower	2.03
S. caprea	Kızılcahamam/Ankara-1	Flower	1.50
S. purpurea subsp. *Leucodermis*	Gökova/Köyceğiz/Muğla-1	Leaf	1.46
S. caprea	Kızılcahamam/Ankara-3	Flower	1.21
S. caprea	NBGT-1	Flower	0.95

[a]*All values were mean of triplicates.*
[b]*Data expressed as grams of gallic acid equivalents per 100 g dry weight (DW).*

Table 3. Total flavonoid content of 20 samples from *S. caprea* and *S. purpurea* subsp. *leucodermis*[a]

Scientific name	Location	Parts	Total flavonoid content (g CE/100g DW)[b]
S. caprea	Kızılcahamam/Ankara-2	Leaf	2.67
S. purpurea subsp. *Leucodermis*	NBGT-1	Bark	2.53
S. purpurea subsp. *Leucodermis*	Gökova/Köyceğiz/Muğla-2	Bark	2.51
S. caprea	Kızılcahamam/Ankara-2	Bark	2.51
S. caprea	NBGT-1	Leaf	2.50
S. purpurea subsp. *Leucodermis*	Gökova/Köyceğiz/Muğla-3	Bark	2.48
S. caprea	NBGT-1	Bark	2.37
S. caprea	Kızılcahamam/Ankara-3	Bark	1.99
S. caprea	Kızılcahamam/Ankara-3	Leaf	1.64
S. purpurea subsp. *Leucodermis*	Gökova/Köyceğiz/Muğla-1	Bark	1.51
S. purpurea subsp. *Leucodermis*	NBGT-1	Leaf	1.46
S. purpurea subsp. *Leucodermis*	Gökova/Köyceğiz/Muğla-1	Flower	1.32
S. caprea	Kızılcahamam/Ankara-1	Bark	1.27
S. caprea	Kızılcahamam/Ankara-1	Leaf	1.26
S. purpurea subsp. *Leucodermis*	Gökova/Köyceğiz/Muğla-1	Flower	1.15

S. purpurea subsp. *Leucodermis*	Gökova/Köyceğiz/Muğla-3	Flower	0.99
S. purpurea subsp. *Leucodermis*	Gökova/Köyceğiz/Muğla-1	Leaf	0.81
S. caprea	Kızılcahamam/Ankara-1	Flower	0.55
S. caprea	Kızılcahamam/Ankara-3	Flower	0.55
S. caprea	NBGT-1	Flower	0.39

[a]*All values were mean of triplicates.*
[b]*Data expressed as grams of catechin equivalents per 100 g dry weight (DW).*

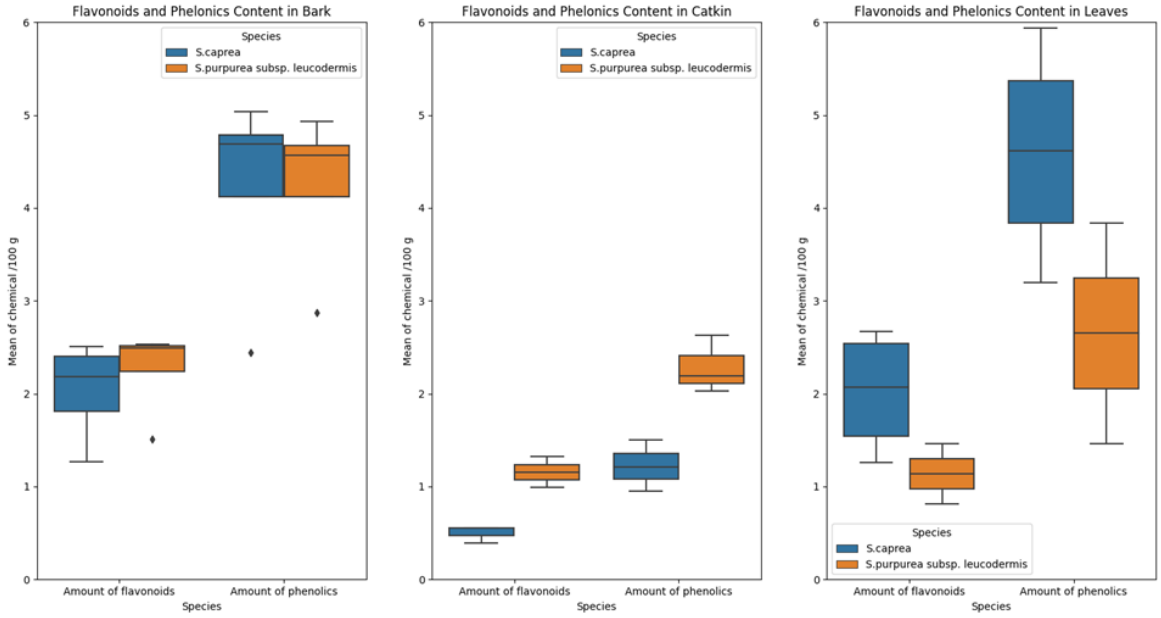

Figure 1. Total phenolic and flavonoid contents of *S. purpurea* subsp. *leucodermis* and *S. caprea* according to organs

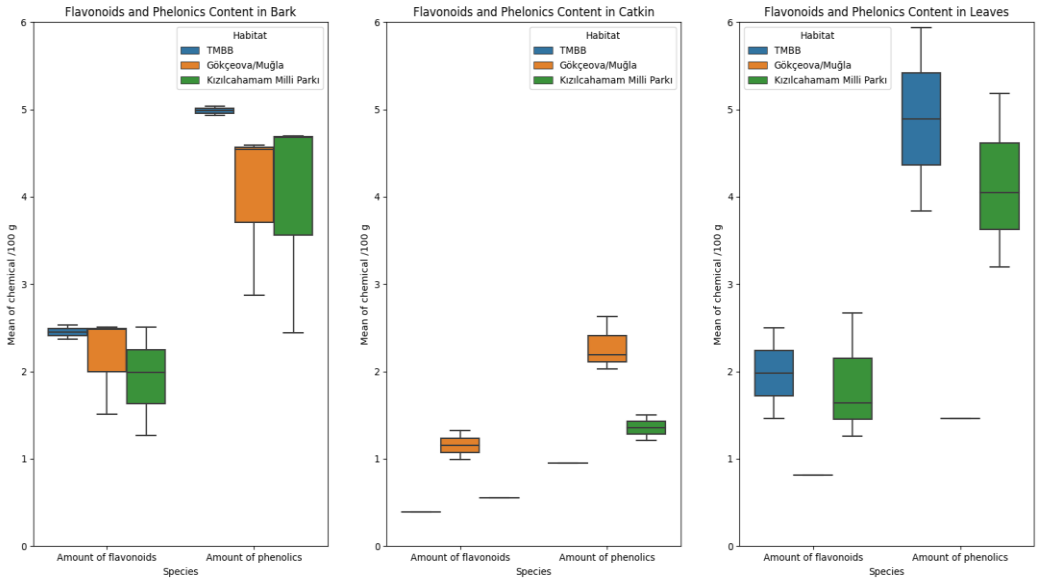

Figure 2. Total phenolic and flavonoid contents of *S. purpurea* subsp. *leucodermis* and *S. caprea* according to habitats

Table 4. DPPH radical scavenging capacity (EC_{50}) of 20 samples from *S. caprea* L. and *S. purpurea* subsp. *leucodermis.*

Scientific name	Location	Parts	EC_{50}[a] (mg)
S. caprea	Kızılcahamam/Ankara-3	Bark	0.25
S. caprea	NBGT-1	Bark	0.31
S. purpurea subsp. *leucodermis*	Gökova/Köyceğiz/Muğla-3	Bark	0.35
S. caprea	NBGT-1	Leaves	0.36
S. caprea	Kızılcahamam/Ankara-2	Leaves	0.36
S. caprea	Kızılcahamam/Ankara-3	Leaves	0.37
S. caprea	Kızılcahamam/Ankara-2	Bark	0.38
S. caprea	Kızılcahamam/Ankara-1	Leaves	0.38
S. purpurea subsp. *leucodermis*	NBGT-1	Bark	0.45
S. purpurea subsp. *leucodermis*	Gökova/Köyceğiz/Muğla-1	Bark	0.56
S. purpurea subsp. *leucodermis*	Gökova/Köyceğiz/Muğla-2	Bark	0.59
S. caprea	Kızılcahamam/Ankara-1	Bark	0.61
S. purpurea subsp. *leucodermis*	NBGT-1	Leaves	0.69
S. purpurea subsp. *leucodermis*	Gökova/Köyceğiz/Muğla-2	Flowers	0.88
S. caprea	Kızılcahamam/Ankara-1	Flowers	1.28
S. purpurea subsp. *leucodermis*	Gökova/Köyceğiz/Muğla-1	Leaves	1.52
S. caprea	Kızılcahamam/Ankara-3	Flowers	1.68
S. caprea	NBGT-1	Flowers	2.62

[a]*EC_{50} is the effective concentration that inhibits DPPH free radical by 50%*

3.3 Antioxidant activity

The highest DPPH radical scavenging activity was detected in the bark in general, especially in the bark of *S. caprea*. Considering the part of the plant used, the leaves had the second-highest DPPH activity, followed by the flowers. In terms of location-based evaluation, it was found that samples mostly from the Kızılcahamam and NBGT had increased DPPH radical scavenging activity. Furthermore, it was discovered that the sample collected from the Gökova/Köyceğiz/Muğla-3 location had a high antioxidant activity for *S. purpurea* subsp. *leucodermis* (Table 4).

4 Discussion

In this study, we examined the phenolic and flavonoid contents, as well as the antioxidant activity, of *S. purpurea* subsp. *leucodermis* and *S. caprea*. Our results indicate that the bark of *S. purpurea* subsp. *leucodermis* and the leaves of *S. caprea* exhibit the highest levels of phenolic and flavonoid compounds, which correlate strongly with their antioxidant potential, as evidenced by DPPH radical scavenging activity. These findings are consistent with earlier studies, such as those by Cai et al. (2004), Sonboli et al. (2010), Zheng and Wang (2001), and Alvar (2024), who highlighted the role of phenolic compounds in the antioxidant activity of various plant species, including those in the Salix genus.

The influence of environmental factors, such as habitat and plant age, was also significant in shaping the chemical composition of these species. Our findings, particularly the high antioxidant activity observed in the NBGT and Kızılcahamam regions, support the idea that habitat and conservation status play crucial roles in the levels of bioactive compounds in plants. This is in agreement with the work of Gligorić et al. (2019), who found that the bark and leaves of *S. caprea* contain significant levels of bioactive compounds. Moreover, Alam et al. (2006) observed similar radical scavenging activity in *S. caprea* flowers, but our study found lower concentrations of phenolics and flavonoids in the flowers compared to the bark and leaves, suggesting that different plant parts contribute differently to antioxidant activity.

Further, the study by Jeppsson (2000) and Tolic et al. (2017) emphasizes that environmental factors, such as climate, soil composition, and conservation practices, have substantial effects on a plant's chemical composition. The high levels of bioactive compounds observed in NBGT can likely be attributed to the young age of the plants, favorable soil conditions, and the ex-situ conservation measures that may have induced stress, leading to an increase in phenolic and flavonoid content, as suggested by Tolic et al. (2017). Alvar (2024) also highlighted the importance of environmental factors in the distribution and bioactivity of *Salix purpurea* subsp. *leucodermis*, further supporting the influence of habitat on plant chemical composition.

Additionally, Akyürek and Acar (2020) discussed the bioactivity and phytochemistry of Turkish Salix species, noting their potential for medicinal use, which aligns with our findings on the high antioxidant potential of *S. purpurea* subsp. *leucodermis* and *S. caprea*. This review

further supports the growing body of literature on the importance of bioactive compounds in the Salix genus, reinforcing the medicinal value of these species.

In conclusion, this study supports the notion that phenolic and flavonoid compounds are key determinants of the antioxidant activity in *S. purpurea* subsp. *leucodermis* and *S. caprea*. The consistency of our findings with those from earlier studies, such as Cai et al. (2004), Sonboli et al. (2010), and Alvar (2024), underscores the importance of these compounds in the medicinal potential of Salix species. Future research should continue to explore the effects of habitat variation, conservation practices, and plant age on the phytochemical composition and bioactivity of these species to better understand their full therapeutic potential.

5 Conclusion

The findings of this study indicate that *S. caprea* (leaves and bark) from the NBGT and Kızılcahamam-2 locations, as well as *S. purpurea* subsp. *leucodermis* (bark) from the NBGT location, exhibit significant antioxidant potential, as evidenced by their high total flavonoid and phenolic content. These results align with previous research, which suggests that salicin alone may not fully account for the antioxidant and anti-inflammatory properties of willow bark. Instead, polyphenolic compounds are likely the primary contributors to these bioactive effects. Our findings further support this hypothesis, emphasizing the importance of *ex-situ* conservation in preserving and studying medicinally valuable plant species. Future research should focus on a more detailed phytochemical characterization of these species to identify the specific compounds responsible for their bioactivity. Additionally, broader geographic sampling and comparative analyses with other willow species could provide further insights into the variability of their antioxidant properties across different environments. Expanding this line of research will be crucial for optimizing the potential applications of *S. caprea* and *S. purpurea* subsp. *leucodermis* in medicinal and pharmacological contexts.

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