

Preliminary Pharmacopoeia Analysis Studies on Endemic *Achillea* Species: *Achillea sipikorensis* Hausskn. & Bornm, and *Achillea lycaonica* Boiss. & Heldr.

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Endemik *Achillea* Türleri *Achillea sipikorensis* Hausskn. & Bornm ve *Achillea lycaonica* Boiss. & Heldr. Üzerinde Ön Farmakope Analizi Çalışmaları

SUMMARY

Achillea is a genus rich in terms of endemic species. *Achillea sipikorensis* Hausskn. & Bornm. and *Achillea lycaonica* Boiss. & Heldr. are two of the *Achillea* species, and they have limited studies on them. In this study, two species were evaluated in terms of some pharmacognostical methods, and the results were compared with each other and with the *Achillea millefolium* species in the pharmacopoeia. The pharmacopoeia analyses were performed as specified in the European Pharmacopoeia 7.0. The antioxidant capacity potential was determined using DPPH and ABTS methods, which are used in the determination of radical scavenging activity. Pharmacopoeia specifications were largely similar to the compared drug. It was examined whether there was radical scavenging activity and antioxidant capacity potential, and it was determined that both plants had high antioxidant capacity potential compared to standard substances. The study revealed the differences and components between the species, and provided an advantage in finding potential areas of use of endemic plants and developing them in the future.

Keywords: *Achillea lycaonica*, *Achillea sipikorensis*, antioxidant activity, Asteraceae.

ÖZ

Achillea, endemik türler açısından oldukça zengin bir cinstir. *Achillea* türlerinden *Achillea sipikorensis* Hausskn. & Bornm. ve *Achillea lycaonica* Boiss. & Heldr. de bunlardan ikisi olup, üzerinde kısıtlı çalışmalar olan türlerdir. Bu çalışmada iki tür bazı farmakognozik yöntemler açısından değerlendirilmiş olup sonuçlar kendi aralarında ve farmakopeye yer alan *Achillea millefolium* türü ile de kıyaslanmıştır. Farmakope analizleri, Avrupa Farmakopesi 7.0'da belirtildiği şekilde gerçekleştirilmiştir. Antioksidan kapasite potansiyeli, serbest radikal temizleme aktivitesinin belirlenmesinde kullanılan DPPH ve ABTS yöntemleri kullanılarak belirlenmiştir. Farmakope spesifikasyonları kıyaslanan droga göre büyük oranda benzerlik göstermiştir. Radikal süpürücü aktivite ile antioksidan kapasite potansiyelinin olup olmadığı incelenmiş, her iki bitkinin de standart maddelere göre yüksek antioksidan kapasite potansiyeline sahip olduğu tespit edilmiştir. Yapılan çalışma ile türler arasındaki farklılıklar ve bileşenleri ortaya koyulmuş, endemik bitkilerin potansiyel kullanım alanlarının bulunmasına ve ileriki süreçte geliştirilmesine avantaj sağlamıştır.

Anahtar Kelimeler: *Achillea lycaonica*, *Achillea sipikorensis*, antioksidan aktivite, Asteraceae.

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INTRODUCTION

Natural resources have been used for centuries in the prevention and treatment of diseases, as well as in diet. When we look at the natural resources used, we come across examples from various plant families. One of the most important and largest plant families, *Asteraceae*, has been used by many societies for years in the prevention and treatment of various diseases. Today, many examples can be given from this family for the most commonly used plants. The *Asteraceae* family is one of the largest flowering plant families with more than 1600 genera and 25,000 species worldwide. Its antioxidant, anti-inflammatory, wound healing, diuretic, etc. effects can be attributed to its components such as flavonoids, terpenes, phenolic acids, and polyphenols. The *Achillea* genus is one of the most important and widely used genera of the *Asteraceae* family, which grows in various regions of the world. The plant, which has been used for many years among the public in the treatment of ailments such as stomachache and cold, has been reported in scientific studies to be due to the phenolic compounds it contains.

The world contains a vast and diverse array of natural resources. Since ancient times, humans have utilized these natural resources in their diets, for protection against diseases and various conditions, and in the prevention and treatment of illnesses. Plants have held significant uses in medicine from prehistoric times to the present day. They are used in their most effective form, combining traditional practices with modern methods. There are many different plant families in the world. One of the largest plant families is the *Asteraceae* family, known to have 25,000 species worldwide. The *Asteraceae* family is widely distributed in various habitats around the world, except for Antarctica. While more common in the Mediterranean, Eastern Europe, and Asia Minor, the *Asteraceae* family has a global distribution. Between 1600 and 1700 genera of plant species exist worldwide in tropical and subtropical climates. Although less com-

mon in tropical areas, they are widely distributed in forests, high-altitude meadows, and even urban green spaces. The *Asteraceae* family is generally known as the sunflower family. *Asteraceae* plants contain the above-ground parts, roots, leaves, fruits, and flowers. They exhibit antioxidant, anti-inflammatory, antipyretic, diuretic, immunomodulatory, antihemorrhoidal, cardiotonic, and anthelmintic bioactivities. These activities are attributed to components such as flavonoids, terpenes, polyphenols, and phenolic acids. The genus *Achillea* L. is one of the genera belonging to the *Asteraceae* family, containing more than 100 species. The *Achillea* genus is mostly distributed in the Middle East, primarily in Iran, but also in Türkiye and Egypt. *Achillea* grows in various parts of Europe, Australia, East and West Asia, North America, and New Zealand. The *Achillea* genus appears to be exceptionally rich in different types of secondary metabolites compared to other plant species. The most common compounds include flavonoids, phenolic acids, terpenes, phytosterols, organic acids, fatty acids, and alcohols. Thanks to these components, they are considered to have analgesic, antipyretic, spasmolytic, cytoprotective, anti-inflammatory, antineuroinflammatory, anthelmintic, antispasmodic, anti-ulcer, antihypertensive, and antihyperlipidemic properties. *Achillea lycaonica* and *Achillea sipikorensis* are plants with potential pharmacological activity that grow in Türkiye (Achika, Arthur, Gerald, & Adedayo, 2014; Koc, Isgor, Isgor, Shomali Moghaddam, & Yildirim, 2015; Nikolić & Stevović, 2015).

Achillea is a genus rich in terms of endemic species. *Achillea sipikorensis* Hausskn. & Bornm. and *Achillea lycaonica* Boiss. & Heldr. are two of the *Achillea* species, and they have limited studies on them. In this study, two species were evaluated in terms of some pharmacognostical methods, and the results were compared with each other and with the *Achillea millefolium* species in the pharmacopoeia. Pharmacopoeia specifications were largely similar to the compared drug. It was examined whether there was

radical scavenging activity and antioxidant capacity potential, and it was determined that both plants had high antioxidant capacity potential compared to standard substances. The study revealed the differences and components between the species, and provided an advantage in finding potential areas of use of endemic plants and developing them in the future.

MATERIALS AND METHODS

Plant material and extraction

The following plant specimens were collected from the Sivas region and identified by Prof. Dr. Mehmet Tekin:

- *Achillea lycaonica* B6 Sivas: Ulaş, near Akkaya village, roadside and surrounding area, 1390 m, M. Tekin 1733.
- *Achillea sipikorensis* B6 Sivas: Sivas-Kangal-Gürün road junction, gypsum hills, 1455 m, M. Tekin 1736.

The root and above-ground parts of both species were appropriately separated, dried, and powdered. The activity study was conducted on the above-ground parts of the plants. Four different types of extracts were prepared from dried and powdered plant samples, ranging from nonpolar to polar solvents. First, 1 L of dichloromethane was added to 250 g of plant material and allowed to macerate for 24 hours (Radovanović et al., 2022). The next day, the plant sample was filtered through filter paper, and 1 L of dichloromethane was added again, repeating the process three times. Then, the three filtrates were combined and evaporated to dryness using a rotavapor. The same procedures were also carried out with ethyl acetate, ethanol, and distilled water, respectively.

Macroscopic and microscopic analysis

Plant specimens were examined under a binocular loupe. The presence of materials that should and should not be present under normal conditions was determined. Under a microscope, powdered above-ground parts of the plant were examined at two different magnifications (10x10 and 10x40) using

Sartur reagent and chloral hydrate solution. The examination was carried out to check the presence and suitability of elements such as stomata-containing epidermal cells, pollen, parenchyma cells, glandular hairs, sclerenchyma cells, and fungal tissue in the plant samples.

Some basic specifications frequently included in the pharmacopoeia monograph

Determination of foreign matter

All drug samples, including the entire contents of the package, were examined for foreign matter as specified in the European Pharmacopoeia 7.0 (Council of Europe, European Pharmacopoeia, 2010). Foreign matter was separated, weighed, and analyzed as a percentage:

$$\text{Foreign matter \%} = (Y \times 100) / X$$

X = Amount of material weighed (g)

Y = Grams of foreign matter found in the weighed material (g)

Loss on drying

Crucibles used as weighing vessels were placed in a 100-105 °C oven for 2 hours, cleaned and dried. After this time, they were transferred to a desiccator and allowed to cool until a constant mass was reached. Tare weights of plant samples were placed in the weighing vessels at 1 g intervals and left in the 100-105 °C oven for 2 hours. After this time, they were transferred to a desiccator and allowed to cool. The cooled weighing vessels were weighed, and the values were recorded. The difference was then calculated as a percentage (Council of Europe, European Pharmacopoeia, 2010). The study was repeated three times.

$$Q = (X + Y) - Z$$

$$\text{Loss during drying \%} = (Q \times 100) / Y$$

X = Tare weight of the weighing vessel

Y = Weight of the plant sample

Z = Weight of the weighing vessel after drying

Q = Mass loss after drying

Total ash determination

Crucibles were placed in a 600 °C ash furnace for 30 minutes, after which they were transferred to a desiccator to cool. Once cooled and reaching a constant weight, the crucibles were weighed, and their tare weight was removed. 1.0 g of plant samples were added to these crucibles and incinerated in a 600 °C ash furnace until completely reduced to ash. After incineration, the crucibles were placed in a desiccator to cool, ensuring they reached a constant weight. Once the crucibles were thoroughly cooled, they were weighed again, and their tare weight was removed (Council of Europe, European Pharmacopoeia, 2010). The study was repeated three times, and the remaining amount was calculated as a percentage:

$$T = Z - X \quad \% \text{ total ash} = (T \times 100) / Y$$

X = Tare weight of the crucible

Y = Gram weight of the plant sample

Z = Mass of the drug-containing crucible transferred to the desiccator after incineration

T = Mass loss after incineration

Determination of ash insoluble in HCl

After the incineration process, 15 mL of hydrochloric acid and 15 mL of distilled water were added to the crucibles containing ash. The crucibles, covered with watch glasses, were left to boil in a water bath under a fume hood for 10 minutes. After cooling, the crucibles were filtered using ash-free filter paper. The filter paper and crucibles were washed with hot distilled water to remove the acid, and then, to evaporate the excess water, the washed filter paper, along with the ash it contained, was placed back into the same crucible and left in the water bath. The crucibles were kept in an ash furnace at 600 °C for 1 hour to burn their contents. They were then transferred to a desiccator to reach a constant weight and cool. The crucibles were weighed, and the amount of ash remaining undissolved in hydrochloric acid was calculated (Council of Europe, European Pharmacopoeia, 2010). The study was repeated three times.

$$\text{Ash insoluble in \% HCl} = 100 \times (A - B) / C$$

A = Weight of the crucible after incineration

B = Tare weight of the crucible

C = Weight of the drug used in total ash determination

Essential oil analysis

A 20 g plant sample was weighed and added to a 1000 mL flask; 500 mL of water was added, and it was brought to a boil in a heater. 0.5 mL of xylene was added to a graduated tube, and distillation could continue for 2 hours. At the end of two hours, the volatile oil collected in the graduated tube was examined (Council of Europe, European Pharmacopoeia, 2010).

Radical scavenging activity tests

DPPH free radical scavenging activity determination

Plant extracts prepared at different concentrations [0, 10, 100, 250, 500, 1000, and 2000 µg/mL] and 20 µL each of ascorbic acid were taken, and 180 µL of 2,2-diphenylpicrylhydrazyl (DPPH) solution (at a concentration of 40 µg/mL) was added. In this experiment, conducted in a 96-well plate, the plates were incubated in a dark environment for 15 minutes, and their absorbances were determined at 540 nm using an ELISA reader. In the control group, ascorbic acid was used as the reference substance without the addition of the test solution. The results were expressed as DPPH scavenging activity % using the following equation. The experiments were performed in triplicate, and the results were evaluated (Clarke, Ting, Wiart, & Fry, 2013).

$$\text{DPPH scavenging activity \%} = (A_{\text{Control}} - A_{\text{Test}}) / A_{\text{Control}} \times 100$$

Control absorbance is solution that does not contain test substances, while test absorbance is the absorbance of the extract/reference substance.

ABTS radical scavenging effect determination

To determine the ABTS scavenging activity of plant extracts, the method described in the literature was used (Re et al., 1999). Test samples were prepared

as in the DPPH method, and the ABTS⁺ radical was prepared by reacting 15 mL of 7 mM ABTS with 264 μ L of 140 mM potassium persulfate solution, which was left to stand in the dark at room temperature for 16 hours before the experiment (stock solution). The ABTS working solution was adjusted to give an absorbance value of $0,70 \pm 0,02$ at 734 nm by diluting the prepared stock solution with methanol. 50 μ L of the test solution was mixed with 100 μ L of the ABTS working solution on a 96-well plate. Plates containing test and butyl hydroxyanisole (BHA) reference solutions prepared at different concentrations (0, 10, 100, 250, 500, 1000, and 2000 μ g/mL) were incubated at room temperature for 10 minutes, and their absorbances were measured at 734 nm. The experiment was performed without adding the test solution to the control group. The ABTS⁺ scavenging activity of the plant extracts was expressed as % ABTS scavenging activity, and the experiments were performed in triplicate.

$$\text{ABTS scavenging activity \%} = (A_{\text{Control}} - A_{\text{Test}}) / A_{\text{Control}} \times 100$$

The control absorbance is solution that does not contain the test substances, and the test absorbance is the absorbance of the extract/reference substance.

RESULTS AND DISCUSSION

Plant specimens were examined in detail under a binocular loupe. When the powdered form of *A. sipikorensis* was examined, mostly greenish-brown leaf, stem, and bark-like structures were observed. A few yellow flowers and buds were also observed within the powder. In addition, spiny, hairy leaves were also observed. The powdered form of *A. lycanica* is similar to *A. sipikorensis*, consisting of greenish-brown tones. Leaf, stem, bark, hairy, and spiny structures were observed in the powder. Microscopic images of *A. lycanica* and *A. sipikorensis* specimens with Sartur and chloral hydrate are given in Figure 1-6.

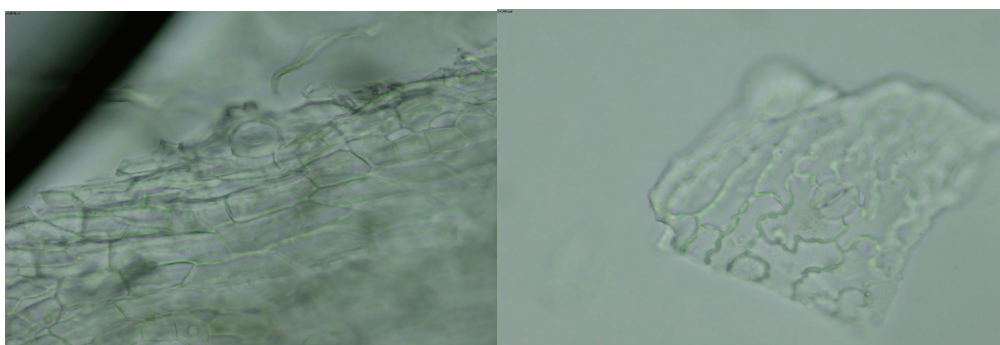


Figure 1. Microscopic image of stomata-bearing stem and leaf epidermal fragments of *A. lycanica*, at 10x40 microscopic magnification; cells with wavy, irregularly thickened walls are placed on a slide prepared with chloral hydrate

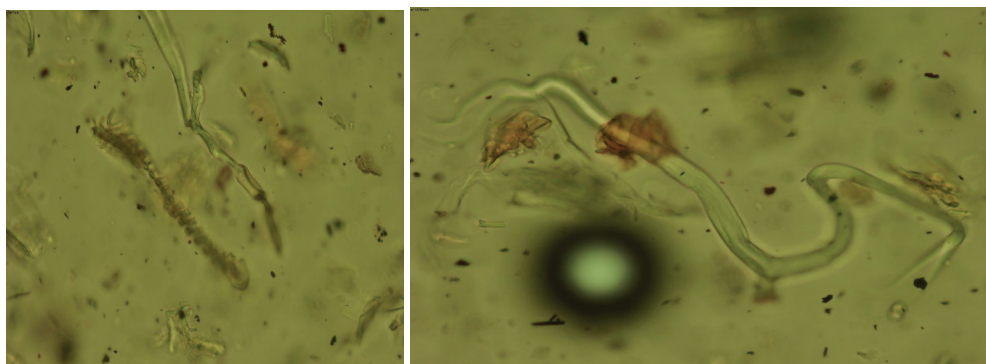


Figure 2. At 10x10 microscopic magnification, the single-row terminal cells of *A. lycanica*, containing thick-walled cells, are surrounded by curved covering hairs

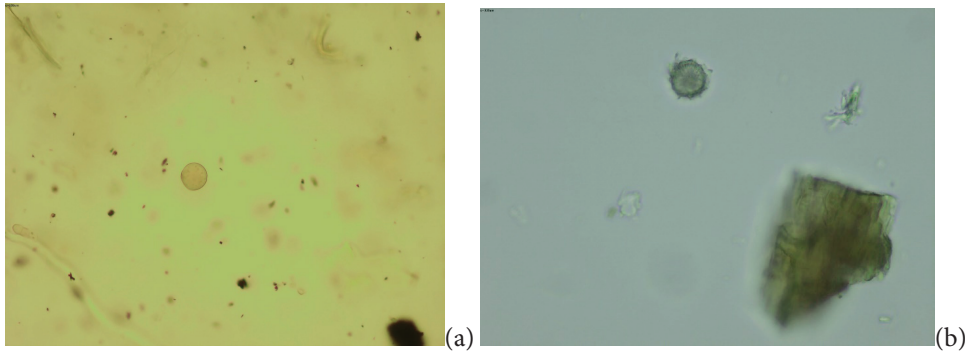


Figure 3. Microscopic image of spherical pollen grains of *A. lycanica* prepared with Sartur (a) and chloral hydrate (b) at 10X40 microscope magnification

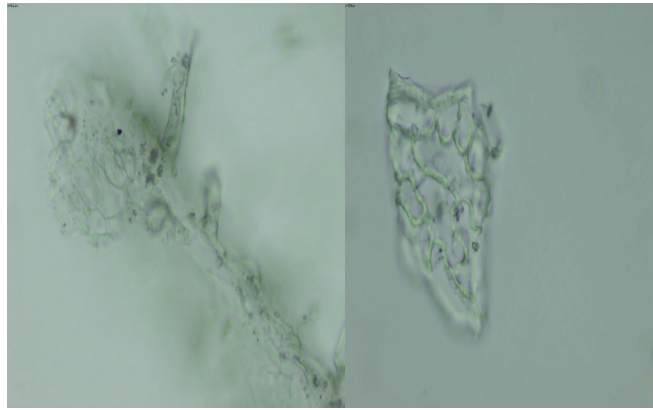


Figure 4. Microscopic image of leaf and bract epidermal fragments of *A. sipikorensis* with wavy, irregularly thickened cell walls, finely striated cuticle and stomata, on a chloral hydrate-prepared slide at 10x40 microscope magnification

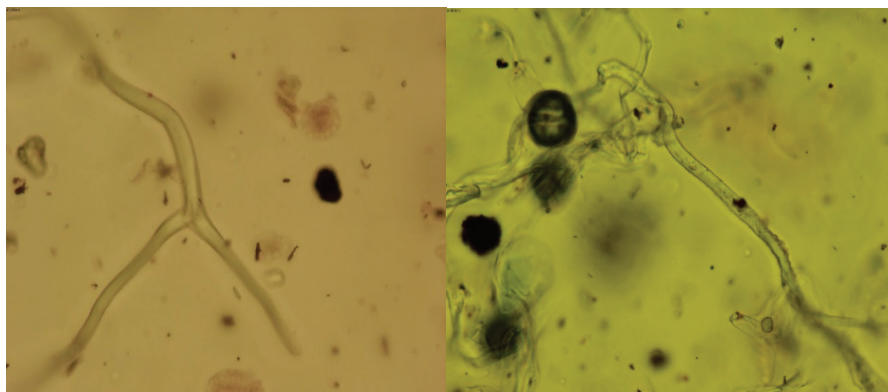


Figure 5. Microscopic image on a slide prepared with Sartur, showing a single row of thick-walled cells of *A. sipikorensis* with curled covering hairs at the tips, at 10x40 microscopic magnification

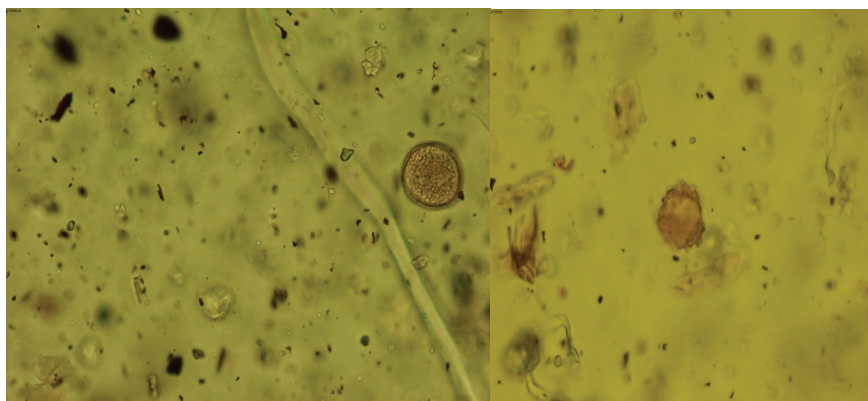


Figure 6. Microscopic image of spiny-walled, spherical pollen grains of *A. sipikorensis* on a slide prepared with Sartur, at 10x40 microscope magnification

Plant samples were examined for foreign matter values as specified in the European Pharmacopoeia 7.0, and the percentage of removed foreign matter was calculated by weighing. The results were compared to the values specified in the European Pharmacopoeia 7.0 for *A. millefolium* drugs, where the maximum percentage of stems with a diameter greater than 3 mm is 5% and the maximum percentage of other foreign matter is 2% (Table 1.).

The results obtained from the treatments, when compared to the maximum loss value of 12% specified in the European Pharmacopoeia 7.0 for *A. millefolium* drugs, were determined to be approximately half of 12% for *A. lycaonica* and *A. sipikorensis* (Table 1.).

After the processing, the results obtained were compared with the total ash content of *A. millefolium*, which can be a maximum of 10% as specified in the European Pharmacopoeia 7.0. It was found that *A. lycaonica* met the pharmacopoeia standards for *A. millefolium*, while the ash content was higher for *A. sipikorensis* (Table 1.).

The essential oil obtained from each material is evaluated in terms of quantity and color. When compared to the 2 mL/kg value specified in the European Pharmacopoeia 7.0 for drugs of the *A. millefolium* plant, the essential oil yield obtained for both *A. lycaonica* and *A. sipikorensis* was found to be high (Table 1.).

Table 1. The results of some basic specifications frequently included in the pharmacopoeia monograph

SAMPLE	Foreign matter %	Loss on drying%	Total ash %	Ash insoluble in HCl %	Essential oil %
<i>A. lycaonica</i>	< %0.5	6.67 ± 0.23	6.03 ± 0.52	1.82 ± 0.98	2.31 ± 0.20
<i>A. sipikorensis</i>	< %0.5	6.82 ± 0.17	18.49 ± 2.42	8.49 ± 0.38	3.94 ± 0.80

According to the results of experiments conducted in triplicate, all extracts prepared from *A. lycaonica* showed higher radical scavenging capacity than those prepared from *A. sipikorensis*. Despite differences in polarity, it is thought that high radical scavenging capacity is present in all extracts, with potentially effective compounds found in the oz derivative form in polar extracts and in the aglycone derivative form

in nonpolar extracts. The dichloromethane extract of *A. lycaonica* had the highest activity, followed by the ethanol extract. ABTS⁺ results showed high radical scavenging results similar to the DPPH method. All extracts prepared from *A. lycaonica* showed higher radical scavenging capacity than those prepared from *A. sipikorensis*. The ethanolic extract of *A. lycaonica* had the highest activity, followed by the aqueous ex-

tract (Table 2.). The lowest DPPH radical scavenging capacity for *A. spikorensis* was found to be $70.11 \pm 1.45\%$ in the ethyl acetate extract at a dose of $10 \mu\text{g/mL}$, while the lowest radical scavenging capacity for *A. lycanica* was found to be $88.57 \pm 1.10\%$ in the aqueous extract at a dose of $10 \mu\text{g/mL}$. The lowest ABTS radical scavenging capacity for *A. spikorensis*

was found to be $82.94 \pm 1.43\%$ in the ethanol extract at a dose of $10 \mu\text{g/mL}$, while the lowest radical scavenging capacity for *A. lycanica* was found to be $85.99 \pm 0.51\%$ in the dichloromethane extract at a dose of $10 \mu\text{g/mL}$. Overall radical scavenging inhibition percentages varied between approximately 70-99%.

Table 2. Radical scavenging activities % of samples in 2 mg/mL concentrations

PLANT SAMPLE/ REFERENCE	EXTRACTION SOLVENT	DPPH Radical scavenging activity (inhibition %)	ABTS ⁺ Radical scavenging activity (inhibition %)
<i>A. lycanica</i>	Dichloromethane	98.35 ± 1.44	98.70 ± 0.30
	Ethylacetate	99.21 ± 0.29	98.18 ± 1.32
	Ethanol	99.82 ± 0.09	99.74 ± 0.11
	Distillated water	96.50 ± 0.74	98.70 ± 1.17
<i>A. spikorensis</i>	Dichloromethane	93.30 ± 1.80	95.83 ± 3.12
	Ethylacetate	94.30 ± 0.45	96.96 ± 0.11
	Ethanol	94.30 ± 0.11	97.55 ± 0.50
	Distillated water	94.30 ± 0.45	98.02 ± 1.55
Ascorbic acid	-	99.71 ± 0.10	-
BHA	-	-	98.69 ± 0.67

In our study with the endemic species *A. lycanica* and *A. spikorensis*, extraction yield, ash content, macroscopic and microscopic analyses, essential oil yield, and antioxidant activity studies were evaluated using the monograph of the *Achillea* species found in the pharmacopoeia as a reference.

As a result of the studies, macroscopic analysis of samples collected from the above-ground parts of both plant species revealed negligible amounts of foreign matter. Microscopic analysis revealed stomata, glandular hairs, pollens, parenchyma and epidermal tissues, and some lignified and fungal tissues from the above-ground parts of the plant. Similar structures have been observed in microscopic analyses of *Achillea salicifolia*, *Achillea santolina*, *Achillea tenuifolia*, and many other species in the literature (Asadullae-va, Ishmuratova, Atazhanova, & Smagulov, 2024; Manayi, Mirnezami, Saeidnia, & Ajani, 2012; Urooj, Mazhar, & Azhar, 2024).

The European Pharmacopoeia 7.0 states that the maximum loss rate during drying of *A. millefolium*

drugs is 12%. When the same study was conducted with four different extracts of *A. lycanica* and *A. spikorensis*, the drying loss percentages were found to be between 6-7%. Considering that these values are below the 12% value specified in the pharmacopoeia for *A. millefolium*, it can be interpreted that these species may have a high potential to meet pharmacopoeia specifications. Ash content determination is carried out to determine whether the drug has been properly cleaned and to obtain information about the amount of inorganic substances, mineral salts, and metals contained in the drug. According to the *A. millefolium* monograph in the pharmacopoeia, the total ash content in 1.00 g of powdered *A. millefolium* drug should not exceed 10%. The data obtained from the study conducted with four different extracts of the two plants showed that the values for *A. lycanica* were between 5-7%, and for *A. spikorensis* between 15-21%. These values will help us interpret the cleanliness of the collected plant samples and the quantities of mineral salts and metals they contain. When compared to the *A. millefolium* value, *A. lycanica*

conforms to the value in the monograph, indicating clean collection and containing fewer mineral salts and inorganic substances; however, the higher value for *A. sipikorensis* suggests that it was not collected cleanly and may contain various inorganic substances or salts. It should be noted, however, that this value is specific to *A. millefolium*. The results obtained, along with our experimental studies and comparative results, have made our study unique, as they contribute to determining whether these values are normal for the two endemic species and to establishing a monograph for these endemic species. The determination of ash insoluble in HCl is another experimental study that will help us gain insight into the inorganic substances in plant samples. In the study of ash insoluble in HCl, the amount of ash remaining in the weighed crucibles was found to be higher than the maximum value of 2% specified in the European Pharmacopoeia 7.0 for the drugs of *A. millefolium*. Considering that the obtained values may be normal or outside the normal values of the two endemic species, more precise conclusions will be reached through further studies. The value specified for essential oil in the monograph of *A. millefolium* is 2 mL/kg. In the three-times repeated essential oil extraction process for *A. lycaonica* and *A. sipikorensis*, the essential oil amounts of *A. lycaonica* were 2.04, 2.49, and 2.41 mL/kg; The essential oil content of *A. sipikorensis* was found to be 2.99, 4.95, and 3.88 mL/kg. It can be said that both plants were obtained with high yields compared to *A. millefolium*. While other studies generally found the essential oil yield of *A. millefolium* to be around 0.025% v/w, another study calculated the essential oil yield of *A. sivasica* Çelik and Akpulat from the above-ground part to be 0.056% v/w. It was also observed that harvesting time, climate, and species differences significantly affected the essential oil percentage (Haliloglu et al., 2017; Öztürk, Kırıcı, & Demirci, 2023). The essential oils obtained from both plants were yellowish-transparent in color. Both visually and in terms of yield, it is thought that the essential oils obtained from these species are

promising for bioactivity and phytochemical studies in future research. Antioxidants are compounds that inhibit specific oxidizing enzymes or can delay or prevent the effects of oxidation. As antioxidants, phenolic compounds prevent the formation of free radicals, which have harmful health effects (Zengin et al., 2017). Therefore, plants with antioxidant capacity potential have the capacity to show beneficial effects against many oxidative stress-related diseases such as cardiovascular disease, cancer, and diabetes. Recent research has shown that the *Achillea* genus possesses antioxidant activity related to its total phenolic and flavonoid content. A study conducted with three different endemic species (*A. pachycephalla*, *A. aucherii* and *A. kellalensis*) growing in Iran showed that these endemic species have acceptable antioxidant activity through DPPH activity studies, proving that they can be used as important natural antioxidant sources for pharmaceutical and medicinal purposes. According to this study, it was found that *A. aucherii*, *A. kellalensis* and *A. pachycephalla* possessed 844 µg/mL, 518 µg/mL and 248 µg/mL IC_{50} values, respectively (Gharibi, Tabatabaei, Saeidi, Goli, & Talebi, 2013). In Türkiye, the antioxidant activities of ethanol extracts of *A. aleppica* subsp. *aleppica*, *A. aleppica* DC subsp. *zederbaueri*, and *A. biebersteinii* have been determined. It has been reported that at the concentration of 250 µg/mL, radical scavenging effects of 85%, 81%, and 73% were observed, respectively, *A. biebersteinii*, *A. aleppica*, and *A. aleppica* DC subsp. *zederbaueri* (Barış et al., 2011). DPPH is an easy and rapid method for evaluating antioxidant activity. The mechanism by which DPPH plays a role in reducing free radicals is based on the hydrogen bonding capacity of some compounds. Some secondary metabolites, such as phenolic compounds, can exhibit antioxidant effects by bonding hydrogen. In this study, free radical scavenging activity determination was performed using ABTS and DPPH for antioxidant activity determination. The results of this study shed light on the potential antioxidant activity of plants. *Achillea* species contain species with potential antioxidant capacity.

In these two endemic species that we studied, ABTS and DPPH activity determinations showed that they have significantly higher potential antioxidant capacity compared to reference substances.

The studies aimed to elucidate various potential properties of two endemic plants, *A. lycaonica* and *A. sipikorensis*. Further research on endemic species is needed. Future research will provide a better understanding of the physicochemical components of these plants, contributing to their potential use in various applications. Plants have been used for centuries to protect against, prevent, and treat various diseases. This study investigates information about the Asteraceae family, one of the largest plant families in the world, used for various purposes since ancient times, and presents information on *A. sipikorensis* and *A. lycaonica*, both belonging to the *Achillea* genus. Based on literature reviews, the botanical information, classifications, uses in traditional and modern medicine, phytochemical components, and biological activities of the Asteraceae family and its two endemic plant species have been examined.

Plant samples for *A. sipikorensis* and *A. lycaonica* were collected from Sivas, and various studies found in the European Pharmacopoeia were conducted to compare and interpret data from the *A. millefolium* monograph (7.0). The characteristics of the two plant species were compared and interpreted as a result of these studies.

There are numerous species, genera, and plant families in the world. The use of plants in traditional and modern medicine has always been fascinating. Studies on these species worldwide continue. Especially the discovery of new endemic plants and research related to these plants is progressing rapidly. Considering the biological activity studies in the literature, different parts of the endemic species *A. sipikorensis* and *A. lycaonica*, which hold an important place among endemic plants, have been observed to have potential for use in medicine,

pharmacy, industry, and food for various purposes. Further research is still needed to understand the various properties of these endemic plants and how to utilize them. Detailed qualitative and quantitative research will help to elucidate the various pharmacological activities of endemic plants, leading to the development of different formulations. These formulations can include tablets, solutions, ointments, etc., as well as potentially medicinal teas that are easily accessible to the public. Phytochemical isolation studies will elucidate the metabolites contained in these plants, shedding light on their biological activities, presenting their potential effects, and contributing to the development of appropriate treatment methods in medicine. With the help of advancing technology, detailed research is being accelerated, and studies driven by interest in the potential properties of plants are increasing. This study highlights the significant potential of endemic plant species in Türkiye, a country with a rich flora, and aims to provide originality and guidance by focusing on more endemic plant species in this field with approaches that do not endanger their survival.

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AUTHOR CONTRIBUTION STATEMENT

Conceptualization, C. D.; Methodology, C.D. and B.Y.; Validation, C.D.; Investigation, B.Y.; Resources, M.T.; Data Curation, C.D.; Writing—Original Draft, B.Y.; Writing—Review & Editing, C.D.; Supervision, C.D. All authors must have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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