Trakya University Journal of Natural Sciences, 26(2), xx-xx, 2025
DOI: 10.23902/trkjnat.54357889
www.tujns.org

Research Article

Antioxidant, antiradical properties, vitamin and trace element status of *Artemisia spicigera* C. Koch distributed in the Türkiye flora

Damla Yıldız[1], Ahmet Bakır[1]*, Suat Ekin[1], Fevzi Özgökçe[2]

[1]*Van Yüzüncü Yıl University, Faculty of Science, Department of Chemistry, Van, Türkiye*
[2]*Van Yüzüncü Yıl University, Faculty of Science, Department of Biology, Van, Türkiye*

Cite this article as: Yıldız, D., Bakır, A., Ekin, S., & Özgökçe, F. (2025). Antioxidant, antiradical properties, vitamin and trace element status of *Artemisia spicigera* C. Koch distributed in the Türkiye flora. *Trakya University Journal of Natural Sciences, 26*(2), xx-xx.

Abstract

It is known that the active substances present in plants contribute to many biochemical processes and provide the balance of organisms. This study aims to determine the antioxidant and antiradical properties of the methanol extract of *Artemisia spicigera (A. spicigera)* C. Koch leaves and to measure the levels of trace elements and vitamins E and K. Trace element analyses were performed by the Inductively Coupled Plasma - Optical Emission Spectroscop dry ashing and vitamin analyses were performed by the High Performance Liquid Chromatography method. Vitamin C, total phenol and flavonoid contencts, antioxidant capacity, DPPH•, ABTS•$^{+}$, O_2•$^{-}$, H_2O_2 and OH• values were determined spectrophotometrically. Total phenolic and flavonoid contents and antioxidant capacity were determined as 18.45 ± 0.39 mgGA/g, 26.29 ± 1.64 mgQE/g and 44.08 ± 2.91 mMAA/g, respectively. Mn, Zn, Cu, α-tocopherol, phylloquinone and ascorbic acid levels were found as 287.32 ± 0.57, 104.36 ± 2.12, 37.40 ± 0.10, 10.28 ± 0.75, 0.149 ± 0.047 µmol/kg and 217.36 ± 29.20 mg/100g, respectively. *In vitro* antioxidant properties determined by DPPH•, ABTS•$^{+}$, H_2O_2 and OH• assays showed maximum inhibitory with respective IC_{50} values of 61.83 ± 0.68, 71.74 ± 0.79, 34.25 ± 0.74 and 77.91 ± 0.88 µg/mL. The IC_{50} values of these assays showed promising antioxidant power. The results obtained in this study showed that the plant leaves migth be a potential candidate for *in vivo* studies to be evaluated in the future. *A. spicigera* can be also used as a potential supplement or in the pharmaceutical industries as a source of natural antioxidants.

Özet

Bitki içeriklerinde bulunan aktif maddelerin pek çok biyokimyasal süreçteki katkıları ve canlı organizmanın dengesini sağladığı bilinmektedir. Bu çalışmada, *Artemisia spicigera (A. spicigera)* C. Koch yaprağının metanol ekstraktının antioksidanve antiradikal özelliklerinin belirlenmesi ve iz element ile vitamin seviyelerinin ölçülmesi amaçlanmıştır. İz element analizleri Endüktif Eşleştirilmiş Plazma – Optik Emisyon Spektroskopisi kuru külleme ile vitamin E ve K analizleri ise Yüksek Performanslı Sıvı Kromatografisi yöntemi ile belirlenmiştir. Vitamin C, toplam fenol ve flavonoid içerikleri, antioksidan kapasite, DPPH•, ABTS•$^{+}$, O_2•$^{-}$, H_2O_2 ve OH• değerleri spektrofotometrik olarak tespit edilmiştir. Toplam fenolik ve flavonoid içerik ile antioksidan kapasitesi sırasıyla 18,45 ± 0,39 mgGA/g, 26,29 ± 1,64 mgQE/g ve 44,08 ± 2,91 mMAA/g olarak belirlenmiştir. Mn, Zn, Cu, α-tokoferol, filokinon ve askorbik asit düzeyleri sırasıyla 287,32 ± 0,57, 104,36 ± 2,12, 37,40 ± 0,10, 10,28 ± 0,75, 0,149 ± 0,047 µmol/kg ve 217,36 ± 29,20 mg/100g olarak bulunmuştur. DPPH•, ABTS•$^{+}$, H_2O_2 ve OH• deneyleri ile belirlenen *in vitro* antioksidan özelliklerin, ilgili IC_{50} değerleri 61,83 ± 0,68, 71,74 ± 0,79, 34,25 ± 0,74 ve 77,91 ± 0,88 µg/mL ile maksimum inhibitör göstermiştir. Bu testlerin IC_{50} değerleri ümit verici antioksidan güç göstermiştir. Bu sonuçlar gelecekte değerlendirilecek *in vivo* çalışmalar için *A. Spicigera* yaprağının potansiyel bir aday olabileceğini kapsamlı bir şekilde göstemiştir. *A. spicigera* aynı zamanda potansiyel bir takviye veya doğal antioksidan kaynağı olarak ilaç endüstrisinde de kullanılabilir.

Keywords: *Artemisia spicigera*, reactive oxygen species, metal, α-tocopherol, phylloquinone

Edited by: *Yeşim Sağ Açıkel*

***Corresponding Author:** Ahmet Bakır, **E-mail:** ahmetbakir@yyu.edu.tr*

***ORCID iDs of the authors:** DY. 0000-0002-9489-3860, AB. 0000-0003-0797-285X, SE. 0000-0002-6502-5028, FÖ. 0000-0002-3119-8561*

Received: 22 January 2025, **Accepted:** 09 May 2025, **Online First**: 07 July 2025,

Introduction

It has long been known that many traditional medicines originate from plants or plant-based sources. The use of plants as medicine is as old as human history and forms the basis of modern medicine (Maridass & De Britto, 2008). According to World Health Organisation data, 25% of the pharmaceutical drugs used today are obtained from plants used for medicinal purposes. Plants produce a large number of secondary metabolites to protect themselves from external attack. These plant-derived compounds pose less of a threat to human health, and with each passing day a new one is being added to the list of plant-derived compounds, providing an alternative to existing synthetic drugs (Boztaş et al., 2021). Recent studies showed that plant components scavenge free radicals formed by oxidative stress and give effective results in the treatment of many diseases occurring in metabolism. It has also been determined that these plant components have fewer side effects than existing drugs (Sen et al., 2010; Rolnik & Olas, 2021).

The health of humans and all other organisms is closely interconnected. Especially antioxidant-derived components obtained from plants offer important natural food support for the protection of health (Gülçin, 2025). It is supported by studies that these herbal supplements applied after oxidative stress-induced diseases (including cancer) are effective by interrupting oxidative chain reactions (Tao et al,. 2020). According to the International Agency for Research on Cancer, in addition to 9.7 million deaths from cancer worldwide, there were approximately 20 million new cancer cases in 2022, and cases are estimated to reach 29.9 million by 2040. For a long time, studies have been carried out to investigate plants to treat cancer naturally and these studies are still up to date (Bajpai et al., 2024). Natural components such as phenols, flavonoids, polysaccharides, which are bioactive components derived from plants and have antioxidant activity, as well as vitamins and elements contained in plants, have strong anticancer effects. Therefore, natural antioxidant drugs obtained from plants can strengthen specific or non-specific immune function. They can also inhibit the production of free radicals by directly preventing cellular tissue damage caused by free radicals (Chank et al., 2020).

The *Asteraceae* family is widely used in traditional medicine for therapeutic applications throughout the world due to its cosmopolitan distribution (Kljucevsek & Kreft, 2025). The presence of flavonoids in this family is remarkable. Members of this family show a wide range of anti-inflammatory, anti-microbial, antioxidant and hepatoprotective activities (Konovalov, 2014). The currently increasing need for natural medicine resources has increased scientific interest in the *Asteraceae* family. Most members of the family are used in traditional medicine both in Türkiye and around the world due to their essential oil production, phytochemical properties and biological activities (Petretto et al., 2013; Rolnik & Olas, 2021). *Asteraceae* is the richest of flowering plants in terms of genus and species diversity. In Türkiye, 133 genera and 1156 species are known to naturally grow, of which 430 are endemic for the flora of the country. In this respect, the endemism rate of the *Asteraceae* in Türkiye is as high as 38%. The genus *Artemisia* belonging to this family includes perennial and economically valuable plants distributed over a wide geographical area, and is represented by 23 species in the flora of Türkiye (Öztürkmen, 1996).

Some species of the *Asteraceae* family (e.g. *A. annua*) have been reported to be anti-bacterial and anti-malarial effects (Soni et al., 2024). To treat drug-resistant strains of the genus *Plasmodium*, either the drug group artemisinin or plants with fewer side effects are recommended (Xiao et al., 2016; Soni et al., 2024). The important phytochemical artemisinin is synthesised in the leaves and flowers of *Artemisia* species and is currently used in the treatment of malaria worldwide (Czechowski et al., 2018). This compound has been shown to be sensitive on some bacterial species such as *Escherichia coli, Staphylococcus aureus* and *Streptococcus faecalis*. Many scientists attach importance to the synthesis of this compound and its derivatives due to increasing drug costs (Peplow, 2018; Ekiert et al., 2022). *Artemisia spicigera (A. spicigera*) C. Koch [bozkır yavşanı (in Turkish)], the endemic used in the present study, is distributed in Armenia, Central Anatolia and Iran (Chehregani et al., 2013). The plant has antiseptic and stomachic properties and is traditionally recommended in Türkiye for the treatment of skin infections, as well as for skin diseases and ulcerative wounds (Abad et al., 2012; Ghorbani et al., 2021).

No study has investigated the relationship between *A. spicigera* and parameters such as antiradical ($DPPH^{\cdot}$, $ABTS^{\cdot+}$, H_2O_2, $OH^{\cdot}$ and $O_2^{\cdot-}$) properties, vitamin (α-tocopherol, phylloquinone and ascorbic acid) and trace element status (As, Be, Cd, Cr, Cu, Sr, Pb, Mo, Li, Mn, Ti, Co, Tl and Zn). However, total phenol, flavonoid contents and antioxidant capacity levels of *A. spicigera* and *A. splendens* plant species collected from Iran region were determined (Afshar et al., 2012).

This study aimed to investigate the free radical scavenging activity of methanol extract of *A. spicigera* leaves by using $DPPH\bullet$, $ABTS\bullet^{+}$, hydrogen peroxide (H_2O_2), hydroxyl ($OH\bullet$) and superoxide ($O_2\bullet$) antioxidant methods. Total phenol, flavonoid content, total antioxidant capacity, vitamins C, E, K and trace element values in dry leaf content were also determined.

Materials and Methods

Plant Materials

The leaves of *A. spicigera* (*Asteraceae)* have been collected in September 2019 in the Saray/Kazlıgöl district of Van city, Türkiye (38° 32' 38.63" N, 44° 12' 17.90" E, at an altitude 2206 m). *A. spicigera* was defined by Prof. Dr. Fevzi Özgökçe. The sample was deposited in Van YYU herbarium, Botany Department, VANF16271. The plant leaf was dried in the shade for a period of two weeks. After drying, the leaves were ground to a fine powder in a super mixer rotary stainless grinder (new nova industrial kitchen equipment, model: Inox SM 108) and stored in coloured glass bottles for analysis.

Leaf Extraction Process

The plant material was pulverized and weighed (20 g), and methanol (MeOH-80%) was added at room temperature (24°C). The plant-MeOH mixture (1:20, g/v) was subjected to the extraction process

for 48 hours to ensure that the pulverized plant dissolves at the highest rate in methanol. Then the mixture was centrifuged at 4.500 rpm for 15 minutes and filtered with a Whatman grade 1 filter paper. Methanol in the filtrate was extracted using a rotary evaporator at 45°C under reduced pressure and the filtrate was removed. The residue remaining in the volumetric flask was lyophilized at -65°C to ensure that the remaining extract was thoroughly removed. It was then dissolved in methanol and made ready for studies. All the steps in the above described procedure were adapted with slight modifications following the method of Cai et al., (2004).

Determination of Trace Elements

The experimental process for trace element determinations was carried out according to the study of Zurera et al., (1987). 1000 mg of the plant sample was placed in a porcelain crucible, in three replicates. 2.1 mL of ethyl alcohol-sulphuric acid solution was added to the samples. The oven was set at 250°C and kept at the same temperature for 1 hour. Then the temperature of the muffle furnace was increased by 50°C every one hour. This process continued until the temperature of the furnace was 550°C. The samples were kept overnight at the final temperature. 5 mL of hydrochloric acid solution was added and made up to 25 mL.The samples were kept for 5-6 hours and then filtered with filter paper and made ready for analysis. Elemental analyses were carried out using the ICP-OES (Thermo iCAP 6300 Due, England) device.

Measurement of Total Phenolic Content

The determination of the total phenol content of the methanol extract of *A. spicigera* leaves was performed using the Folin-Ciocelteu (FCR) indicator (Yi et al., 1997; Gamez-Meza et al., 1999). 300 µg of 2% sodium carbonate was added to the prepared samples, then 100 µL of FCR indicator was added and incubated for 2.5 hours at room temperature. The absorbance of the samples was read against the control sample at 765 nm. A standard curve was constructed using different concentrations of gallic acid solution. The total phenol content of the plant was reported in milligrams of gallic acid equivalent (GAE) per gram of dried extract (mg GAE/g).

Measurement of Total Flavonoid Content

The extract prepared for the determination of the total flavonoid content of *A. spicigera* was diluted with methanol and used. 500 µL of this diluted solution was taken and 100 µL potassium acetate, 100 µL aluminium nitrate and 4.6 mL ethanol were added and vortexed. The mixture was kept for 45 min at room temperature. The absorbance of the samples was read against the control sample at 415 nm (Lamaison et al., 1990). Flavonoid concentration was calculated by plotting quercetin and the total flavonoid content of the samples was reported as quercetin equivalent (mg QE/g).

Measurement of Total Antioxidant Capacity

The measurement of the total antioxidant capacity of *A. spicigera* was based on the study of Prieto et al., (1999). The prepared plant extract was diluted with methanol and 0.2 mL was taken for each sample. 2 mL of marker solution [0.6 M H_2SO_4 + 28 mM Na_2HPO_4 + 4 mM $(NH_4)_2MoO_4$)] was added to the samples and kept in a 100°C water bath for 90 min. Samples were read against the control at the appropriate wavelength. A standard graph was drawn using ascorbic acid solution and used for total antioxidant capacity calculation. Total antioxidant capacity is given as mM ascorbic acid/g.

Scavenging Activity of DPPH• Free Radical

In this method, extracts of different concentrations were placed in test tubes. 5 mL of DPPH (0.004%) solution was added to 200 µL of sample and incubated in the dark for 30 minutes. The absorbance values of the samples were read against the control sample (DPPH) at 517 nm and recorded. The inhibition percentages of the samples were calculated by Equation 1. BHT was used as positive control value. The obtained % inhibition values were plotted against the concentration. Then, the 50% inhibition values (IC_{50}) of the plant extract against DPPH• radical was determined (Cuendet et al., 1997; Chen et al., 2009).

Equation 1

$$\text{Inhibition (\%)} = \left\{ \frac{A_{Blank} - A_{Sample}}{A_{Blank}} \right\} X\ 100$$

Scavenging Activity of Superoxide ($O_2\bullet^-$) Free Radical

This method is based on measuring the absorbance of NBT at 560 nm with a spectrophotometer (Zhishen et al., 1999). 0.05M phosphate buffer (pH: 7.8) was prepared. 200 µL riboflavin, 0.2 mL L-methionine and 0.2 mL NBT were added to the samples. The samples were kept under fluorescent light for 40 minutes at room temperature. Absorbance was measured at 560 nm against the control sample and compared with BHT. The % inhibition values of the samples were determined using Equation 2.

Equation 2

$$\text{Inhibition (\%)} = \left\{ \frac{A_{Blank} - A_{Sample}}{A_{Blank}} \right\} X\ 100$$

Hydrogen Peroxide (H_2O_2) Radical Scavenging Activity

At 230 nm, the absorbance values of the samples were read against the control sample (Ruch, 1989). 43 mM H_2O_2 solution was prepared using phosphate buffer (pH: 7.4). Plant samples were taken at different concentrations. BHT solution was used as the standard antioxidant substance. The volume of the BHT solution was completed to 0.4 mL with buffer solution. Then 0.6 mL of H_2O_2 solution was added and the mixture was kept at room temperature for 10 minutes. After the waiting time, absorbance values were read and recorded at 230 nm against the control sample (buffer solution). Inhibition values were calculated according to Equation 3.

Equation 3

$$\text{Inhibition (\%)} = \left\{ \frac{A_{Blank} - A_{Sample}}{A_{Blank}} \right\} X\ 100$$

Hydroxyl Radical (OH•) Scavenging Activity

In this method, OH• radical was formed by EDTA/Fe^{+3}/ascorbate/H_2O_2 system and absorbance was measured at 532 nm with spectrophotometer by using the competitive reaction property of deoxyribose (Kunchandy & Rao, 1990).

Stock samples were added to solutions of 2.8 mM deoxyribose, 1 mM $FeCl_3$, 1 mM EDTA, 1 mM ascorbic acid and 1 mM H_2O_2. 20 mM phosphate buffer (pH: 7.4) was prepared. This phosphate buffer was added to the samples to a final volume of 1 mL. The samples were kept in an oven at 37°C for 1.5 hour. Then 1000 µL TBA and 1000 µL TCA were added and vortexed, and the mixture was kept in an oven at 100°C for 30 minutes. The absorbance values of the samples were read against the control sample at 532 nm and recorded. The % inhibition values were plotted and IC_{50} values were determined. The obtained data were compared with BHT and inhibition values were calculated according to Equation 4.

Equation 4

$$\text{Inhibition (\%)} = \left\{ \frac{A_{Blank} - A_{Sample}}{A_{Blank}} \right\} X\ 100$$

Scavenging Activity of ABTS•$^+$ Free Radical

The methanol extract of *A. spicigera* leaves was carried out according to the method of Pellegrini et al., (1999) with a slight modification. $ABTS^{-+}$ radical was prepared in 2.45 mM potassium persulfate and 2 mM ABTS phosphate buffered solution. These two solutions were then mixed and kept at room temperature in darkness for 12-16 hours to form $ABTS^{-+}$ cation radical. $ABTS^{-+}$ cation radical was diluted appropriately and its absorbance at 734 nm was adjusted to 0.70-0.75 and used in the experiment. The radical was then added to stock solutions prepared at different concentrations. Activity (%) was calculated using Equation 5. The plant's scavenging power against stable free radicals was performed with a synthetic antioxidant, trolox.

Equation 5

$$\text{Inhibition (\%)} = \left\{ \frac{A_{Blank} - A_{Sample}}{A_{Blank}} \right\} X\ 100$$

Vitamin C (Ascorbic Acid) Analysis of *A. spicigera* Extract

In order to determine the vitamin C content of *A. spicigera* leaves, vitamin C was measured at 521 nm wavelength using ultraviolet-visible spectrophotometric method. 500 mg of the plant sample was taken and placed in plastic tubes. The experiment was performed in three replicates. 2 mL of HPO_3 acid and 0.5% oxalic acid were added to the tubes and centrifuged at 5000 rpm for 5 min. 2 mL of the filtrate was taken and 0.05 mL thiourea and 0.5 mL 2,4 dinitrophenylhydrazine were added. The samples were then placed in a 90°C water bath for 6 minutes. Then 2 mL of sulphuric acid solution was slowly added to the samples placed in an ice bath. After reaching the appropriate temperature, the samples were vortexed and their absorbance was measured against the control sample. The recorded ascorbic acid concentration values were calculated from the calibration graph (Golubkina et al., 2018).

Vitamin E (α-tokoferol) and K (Phylloquinone) Analysis of *A. spicigera* Extract by High Performance Liquid Chromatography

For phylloquinone and α-tocopherol, a stock solution was prepared at 500 µg/mL, diluted with methanol as appropriate. Linear regression analysis of the peak area versus standard solution concentrations was used to calculate the calibration.

The amount of phylloquinone and α-tocopherol in *A. spicigera* leaves was determined according to the method described in the literature (Sahin et al., 2005; Al-Saleh et al., 2006). For the analysis, 300 mg of the plant was weighed. Hexane-ethanol (50:30) solution was added and kept overnight. The samples were filtered and taken into 1 mL tubes (this procedure was repeated three times). 0.01% BHT was added in each tube, the tubes were vortexed and kept in the dark for 25 hours. Then they were centrifuged at +4°C and 5000 rpm for 15 min. The supernatant was filtered using a filter paper and 500 µL n-hexane was added and dried at 37°C under nitrogen gas. After drying, the residue was dissolved in 500 µL MeOH + C_4H_8O (98%) and prepared for analysis.

Phylloquinone and α-tocopherol analyses were performed on a Gl Science C18 reversed phase HPLC column (250 x 4.6 mm ID), methanol (80 mL) + tetrahydrofuran (20 mL) mobile phase, 1500 µL/min flow rate at 24°C. HPLC (brand: Agillent, model: 1100) - applications were performed at 248 nm phylloquinone and 290 nm α-tocopherol in 0.1 mL volumes in dark bottles in a tray autosampler (-10°C) using a photodiode array detector. Chromatographic analysis was performed at 40°C by isocratic elution.

Statistical Analysis

Experiments were performed with three replicates of each treatment measurements. Means and standard errors of the data were shown as X ± SEM. Group plots were constructed by finding X ± scanning electron microscope. IC_{50} values were determined using nonlinear regression analysis. The statistical analysis were performed by the software SPSS v23 (Chicago, USA).

Results

Folin-Ciocalteu reducing capacity [Folin-Ciocalteu Reagent (FCR) Assay], flavonoid content, total antioxidant capacity, vitamins and trace elements were evaluated to determine the antioxidant properties of methanol extract of *A. spicigera* leaves and results are shown in Table 1. 1,1-diphenyl-2-picrylhydrazyl (DPPH•)

radical scavenging, 2,2¢-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical (ABTS•$^+$) scavenging, hydrogen peroxide (H_2O_2), superoxide radical anion (O_2•$^-$), hydroxyl radical (OH•) scavenging % inhibition and IC_{50} (µg/mL) values compared to the positive control (BHT and trolox) are presented in Table 2.

Total phenol, flavonoid content and total antioxidant capacity values of methanol extract of *A. spicigera* leaves are shown in Figure 1. The % inhibition and IC_{50} values of *A. spicigera* against DPPH•, ABTS•$^+$, H_2O_2, OH• and O_2•$^-$ radicals after comparison with standard antioxidant (BHT and trolox) are shown in Figures 2-6.

The levels of As, Be, Cd, Co, Mo, Pb and Tl and phylloquinone measured in the dry leaves of A. spicigera are shown in Figure 7, and of Cu, Mn, Li, Sr, Ti, Zn and Cr, α-tocopherol and ascorbic acid are shown in Figure 8.

Discussion

If there are too many free radicals in the cell or if the protection provided by antioxidants is low and inadequate, oxidative stress develops in a way that can lead to chronic and permanent damage (Abdollahi et al., 2004). In this case, oxidative stress causes tissue damage in the cell and as a result, many diseases such as cardiovascular and diabetes occur (Tao et al., 2020; Block et al., 2002).

Natural antioxidant compounds have pharmacological potential as they have little or no side effects. Plants are a source of natural antioxidants that prevent oxidative stress. The most important natural antioxidants are flavonoids, tocopherols and phenolic acids. This is why antioxidant-containing compounds can help prevent many diseases, especially those associated with ROS (Kafshboran et al., 2011). The important flavone compound of *A. spicigera* species is hispidulin. This compound is known as anti-cancer (Chaudhry et al. 2024).

α-Tocopherol, phylloquinone and vitamin C contents of *A. spicigera* were evaluated. α-tocopherol exhibits significant antioxidant activity. Considering that *A. spicigera* has not been examined in terms of vitamin parameters in any study conducted to date, these data will make a significant contribution to the literature. In addition, the FCR assay, flavonoid content and total antioxidant capacity of the plant leaf were measured (Table 1 and Figures 1, 7, 8).

In this study, the activity of methanolic extracts (40% MeOH-water) of *A. spicigera* and *A. splendens* growing in Iran was investigated and the total phenolic content of *A. spicigera* was determined as 33.69 ± 1.49 in mg/100 g, total flavonoid content as 96.41 ± 8.74 in mg/100 g, and antioxidant activity (RC_{50}) as 0.0121 ± 0.003 in mg/L. Kafshboran et al., (2011) investigated the radical scavenging activity of *A. spicigera* according to its flavonoid content and when the total phenol and flavonoid contents of *A. spicigera* plants collected from different regions were compared, it was found that *A. spicigera* plants collected from Sufian regions had higher total phenol and flavonoid contents. When the results are compared

Table 1. Vitamins E, C and K, and trace element (As, Be, Cd, Cr, Cu, Sr, Pb, Mo, Li, Mn, Ti, Co, Tl, Zn) levels, and total phenolic and flavonoid content, and antioxidant capacity in leaves of *Artemisia spicigera*.

Parameters	**$\bar{X}$ ± SEM**
α-tocopherol (µmol/kg) (y = + 2.3645 18.3313x; r^2 = 0.9949) Vitamin C (mg 100/g) (y = 0.1268 + 0.1267x; r^2 = 0.9889) Phylloquinone (µmol/kg) (y = + 16.093 101.33x; r^2 = 0.9783)	10.28 ± 0.75 217.36 ± 29.20 0.149 ± 0.047
Total phenolic content (mg GA/g)	18.45 ± 0.39
Total flavonoid content (mg QE/g)	26.29 ± 1.64
Total antioxidant capacity (mM AA/g)	44.08 ± 2.91
As (mmol/kg)	1.22 ± 0.01
Be (mmol/kg)	2.31 ± 0.12
Cd (mmol/kg)	0.47 ± 0.006
Cr (mmol/kg)	7.66 ± 0.21
Cu (mmol/kg)	37.40 ± 0.10
Sr (mmol/kg)	110.40 ± 0.66
Pb (mmol/kg)	1.74 ± 0.59
Mo (mmol/kg)	2.18 ± 0.035
Li (mmol/kg)	155.14 ± 0.49
Mn (mmol/kg)	287.32 ± 0.57
Ti (mmol/kg)	54.59 ± 0.83
Co (mmol/kg)	0.95 ± 0.008
Tl (mmol/kg)	0.31 ± 0.085
Zn (mmol/kg)	104.36 ± 2.12

GA = gallic asid; QE = quercetin; AA = ascorbic acid; SEM = scanning electron microscopy. Values are expressed as ± SEM). All values are mean ± standard deviation of three replicates.

Table 2. Values of % inhibition and IC_{50} (µg/mL) in methanol extract of leaf of *Artemisia spicigera* compared with a positive control.

	Control	**% inhibition ($\bar{X}$ ± SEM)**	**IC_{50} (µg /mL) ($\bar{X}$ ± SEM)**
DPPH•		89.55 ± 0.04	61.83 ± 0.68
	BHT	83.65 ± 0.29	49.58 ± 0.54
ABTS•$^+$		90.03 ± 1.85	71.74 ± 0.79
	Trolox	93.71 ± 0.04	26.23 ± 0.36
H_2O_2		53.93 ± 0.22	34.25 ± 0.74
	BHT	55.59 ± 0.24	19.92 ± 0.37
OH•		65.79 ± 0.33	77.91 ± 0.88
	BHT	77.12 ± 1.02	103.40 ± 234
O_2•$^-$		69.88 ± 0.43	
	BHT	63.11 ± 2.88	

SEM = scanning electron microscopy.

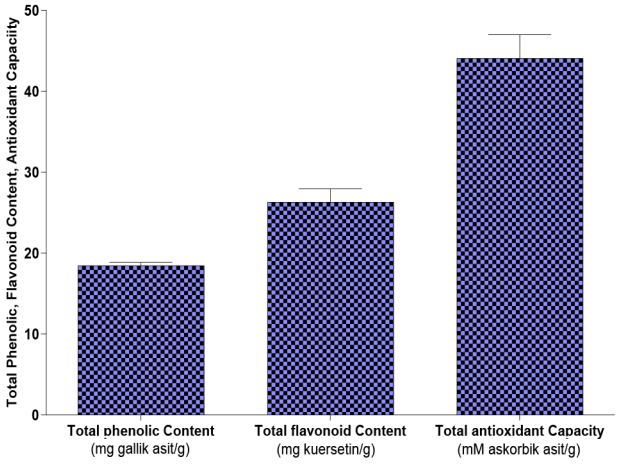

Figure 1. Total phenol, flavonoid content and total antioxidant capacity of *A. spicigera.*

as shown in Table 1, it was determined that the folin-ciocolteu reducing capacity and phenolic content values were lower in our study, but the total antioxidant capacity was very high (44.08 ± 2.91 mM AA/g). The level of active content of each plant may definitely differ depending on the family it is in, the soil structure where it grows, its geographical location, and the climate it is in.

The elemental composition of plants is an important quality parameter that shows the degree of safety of plants and the effect of mineral content on their biological activity. The elements present in the structure of the plant are also important as they will affect the drugs to be obtained from the plant (Golubkina et al., 2018; Chaachouay & Zidane, 2024).

In our current study, trace elements levels of *A. spicigera* were determined and the values found are shown in Table 1 and Figures 7, 8. When the results obtained were compared, it was observed that there was a degreasing relationship between Mn > Li > Sr > Zn > Ti > Cu > Cr > Be > Mo > Pb > As > Co > Cd > Tl. Golubkina et al., (2018) determined the presence of 26 elements in *A. abrotanum,*

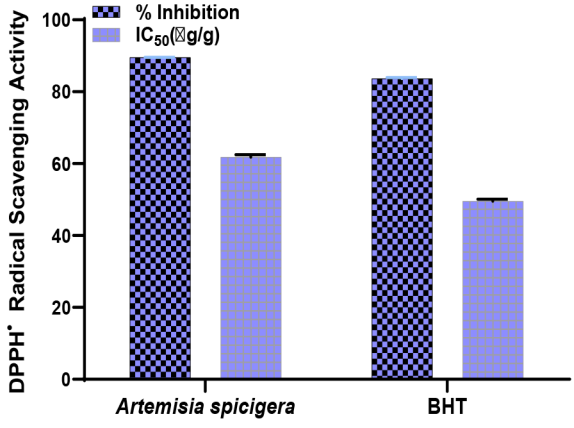

Figure 2. % inhibition of DPPH• radical and IC_{50} value for *A. spicigera* and standard antioxidant BHT.

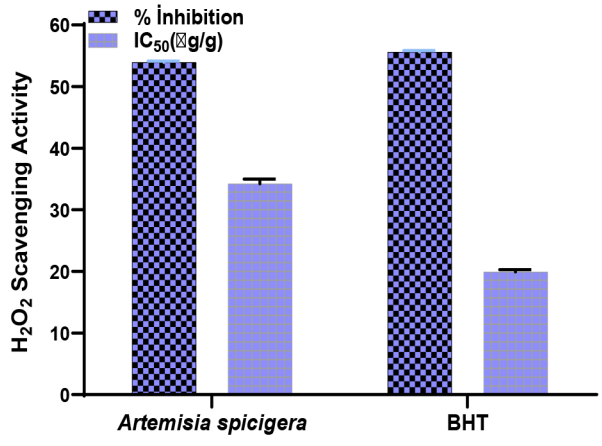

Figure 4. % inhibition of H_2O_2 radical and IC_{50} value for *A. spicigera* and BHT.

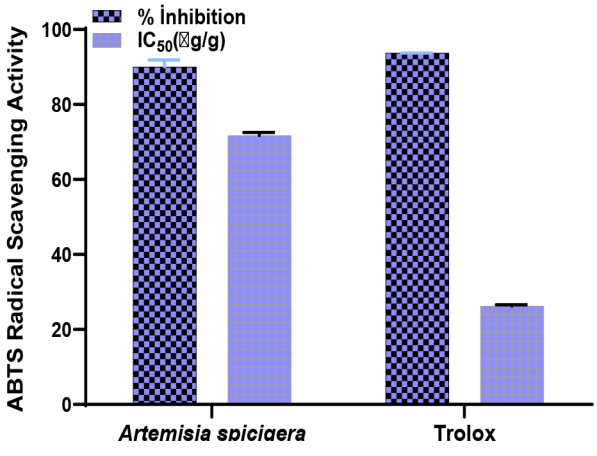

Figure 3. % inhibition of ABTS•$^+$ radical and IC_{50} value for *A. spicigera* and standard antioxidant trolox.

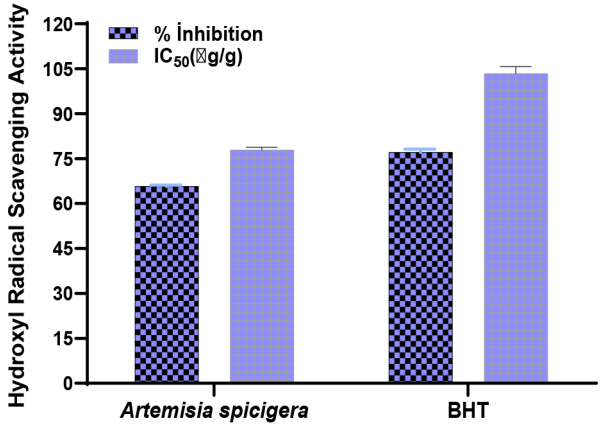

Figure 5. % inhibition of OH• radical and IC_{50} value for *A. spicigera* and BHT.

A. feddei, A. taurica willd, A. dranculucus, A. annua, A. santonica and *A. scoporia* by the ICP-MS method. The presence of Al, Cr, Pb, As and V as heavy metals in *A. scoparia* and Mn, I, Zn and Cu in *A. annua* were determined. In addition, a high Na content was determined in *A. santonica*. However, differently, the presence of trace elements such as Mo, Mn and Co was detected in *A. spicigera.*

In our study, the anti-radical capacity of *A. spicigera* was evaluated by measuring the DPPH• radical scavenging avtivity and the inhibition percentage and IC_{50} concentration results are given in Table 2 and Figure 2. The lower the IC_{50} value, the higher the anti-radical capacity. IC_{50} is the concentration of a substance required to inhibit a specific biological or biochemical function by 50%. On the other hand, this value gives us an idea of how effective a substance is at inhibiting a target and also allows us to compare biological activity between different compounds under the same conditions (Marinova & Batchvarov, 2011; Gülçin & Alwasel, 2023). According to our results, the methanolic extract of leaves of *A. spicigera* showed significant radical scavenging activity showing a value close to BHT (IC_{50} 61.83 µg/mL).

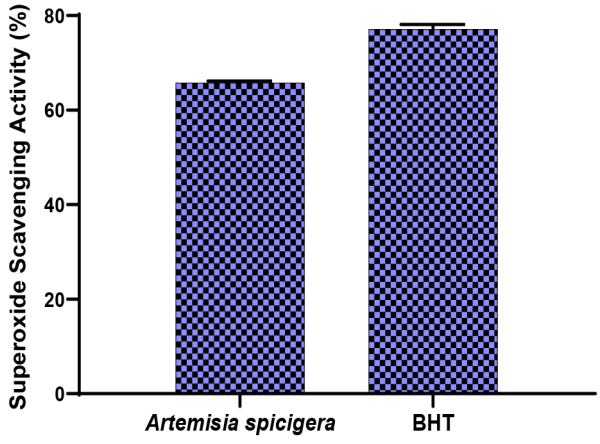

Figure 6. Values of % inhibition of O_2•$^-$ radical for *A. spicigera* and BHT.

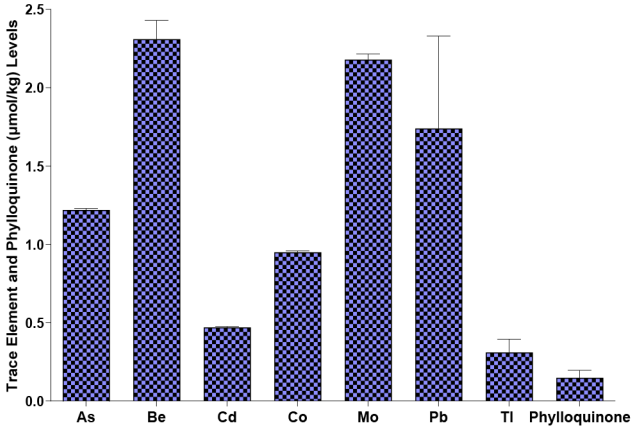

Figure 7. Trace element (As, Be, Cd, Co, Mo, Pb and Tl) and phylloquinone levels in the dry leaves of *A. spicigera.*

Lee et al., (2015) conducted a study on the measurement of DPPH• radical scavenging activity of *A. annua, A. feddei, A. apiacea, A. argyi, A. japonica, A. capillaris, A. gmelinii, A. japonica* ssp. *littoricola, A. keiskeana, A. montana, A. indica, A. rubripes, A. selengensis, A. sieversiana* and *A. stolonifera*. IC_{50} (µg/mL) values for these species were 190.54 ± 2.80, 71.70 ± 4.53, 36.32 ± 4.92, 33.05 ± 1.12, 46.41 ± 7.60, 448.15 ± 78.48, 71.41 ± 2.90, 59. 27 ± 1.51, 74.31 ± 6.42, 18.10 ± 2.04, 60.11 ± 4.67, 38.00 ± 1.27, 54.25 ± 0.96, 336.75 ± 18.34 and 6.64 ± 0.34, respectively. When the DPPH• radical scavenging activity of these species was compared with *A. spicigera,* it was observed that *A. spicigera* showed better radical scavenging activity than approximately 50% of the plants studied.

Kafshboran et al. (2011) investigated the DPPH• radical scavenging activity of *A. spicigera* collected from 5 different regions of East Azerbaijan. Compared to our study, *A. spicigera* (IC_{50} 61.83 ± 0.68) collected from Sufian (ASS, IC_{50} 32.18), Ahar (ASA, IC_{50} 29.74) and Khaje (ASK, IC_{50} 58.93) regions had lower DPPH• radical scavenging activity than the species collected from Dare-diz (ASD, IC_{50} 62.94) and Julfa (ASJ, IC_{50} 64.18) regions. In general, it was observed that there was a consistent with the plants collected in 3 regions and the region we collected.

In the presence of H_2O_2 and O_2•$^-$ radicals OH• radical, which is highly reactive and harmful among free radicals, is formed (Tumilaar et al., 2024). The very short half-life of the OH• radical causes it to react rapidly with some molecules such as sugars, amino acids, nucleic acids, making this radical dangerous. So much so that it causes serious damage to proteins, carbohydrates, DNA and lipids (Sen et al., 2010).

In our study, H_2O_2, OH• scavenging and O_2•$^-$ values in methanol extract of *A. spicigera* leaves were determined. High inhibition percentages and IC_{50} values of H_2O_2 and OH• are given in Table 2 and Figures 4 and 5. In addition, the inhibition percentage of O_2•$^-$ radical anion is shown in Figure 6. The results showed that the H_2O_2 radical scavenging activity of *A. spicigera* was lower than that of BHT, while its OH• radical scavenging activity. The scavenging activity of the plant for O_2•$^-$ radical was also more effective compared to BHT.

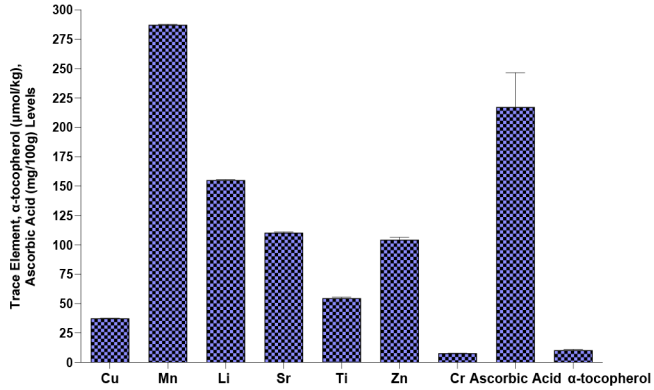

Figure 8. Trace element (Cu, Mn, Li, Sr, Ti, Zn and Cr) levels, ascorbic acid and α-tocopherol levels in the dry leaf of *A. spicigera.*

ABTS•$^{+}$ is not a radical but reacts with potassium persulfate or sodium persulfate to form ABTS•$^{+}$ radical. This radical is dark blue/green in colour and is quite stable. Through this radical, the antioxidant capacity of the solutions can be determined. In the presence of antioxidant, dark blue/green colour lightening occurs (Erel, 2004). We investigated the ABTS•$^{+}$ radical scavenging activity of *A. spicigera*. The highest inhibition percentage of the methanol extract was 90.03 ± 1.85, while it was 93.71 ± 0.04 for trolox. Fifty per cent inhibition of ABTS•$^{+}$ radical was determined as 71.74 ± 0.79 µg/mL for *A. spicigera* and 26.23 ± 0.36 µg/mL for trolox (Table 2 and Figure 3). When the results were examined, it was determined that the percentage inhibition values of the plant and trolox were very close to each other and the plant had a radical scavengingactivity. In the study conducted by Lee et al., (2015) on the measurement of ABTS•$^{+}$ radical scavenging activity of *A. annua, A. japonica ssp. littoricola, A. apiacea, A. argyi, A. capillaris, A. feddei, A. gmelinii, A. japonica, A. keiskeana, A. montana, A. indica, A. rubripes, A. selengensis, A. sieversiana* and *A. stolonifera* were determined as 74.46 ± 1.12, 38.27 ± 1.93, 19.22 ± 0.37, 11.93 ± 0.62, 115.80 ± 3.61, 46.59 ± 4.21, 60.34 ± 2.84, 28.55 ± 1.30, 44.99 ± 1.58, 12.75 ± 0.26, 35.86 ± 2.60, 29.82 ± 0.21, 27.50 ± 2.25, 232.39 ± 12.22 and 15.39 ± 0.24, respectively. When the studies were compared, it was observed that the ABTS•$^{+}$ radical scavenging activity of *A. spicigera* plant used in the study showed lower radical scavenging activity compared to other plant species.

Conclusion

The results obtained showed that the vitamin, total phenol, flavonoid and antioxidant capacity levels and trace element ratios of *A. spicigera* were at a significant level. The methanol extract of *A. spicigera* leaves showed a better antioxidant power compared to the synthetic antioxidant of OH• and O_2•$^{-}$ radicals. In the same way, it was observed that the plant extract had scavenging effects on DPPH• and ABTS•$^{+}$ radicals, but lower than the synthetic antioxidants used for comparison. The fact that *A. spicigera* is used as a spice in food and also as a natural remedy for many diseases develops the idea that this plant has antioxidant properties, and the data obtained here are in parallel with this idea. This plant can be also used as a potential supplement or in the pharmaceutical industries as a source of natural antioxidants.

Ethics

Ethics Committee Approval: Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

Data Sharing Statement: All data are available within the study.

Footnotes

Author Contributions: Conceptualization: D.Y., A.B., S.E.; Design: D.Y., A.B., S.E.; Execution: D.Y., A.B., S.E.; Material Supplying: D.Y., A.B., S.E., F.Ö.; Data Acquisition: D.Y., A.B., S.E.; Data Analysis/ Interpretation: D.Y., A.B., S.E.; Writing: D.Y., A.B., S.E.; Critical review: D.Y., A.B., S.E.

Conflict of Interest: The authors have no conflicts of interest to declare.

Funding: The study was supported by the Van Yüzüncü Yıl University Scientific Research Projects Coordination Unit with project number 2022/

FDK-9894.

References

Abad, M. J., Bedova, L. M., Apaza, L., & Bermejo, P. (2012). The *Artemisia L.* genus: a review of bioactive essential oils. *Molecules, 17*, 2542–2566. https://doi.org/10.3390/molecules17032542

Abdollahi, M., Ranjbar, A., Shadnia, S., Nikfar, S., & Rezaie, A. (2004). Pesticides and oxidative stress: a review. *Medical Science Monitor, 10*(6), 141–147.

Afshar, F. H., Delazar, A., Nazemiyeh, H., Esnaashari, S., & Moghadam, S. B. (2012). Comparison of the total phenol, flavonoid contents and antioxidant activity of methanolic extracts of *Artemisia spicigera* and *A. splendens* growing in Iran. *Pharmaceutical Sciences, 18*(3), 165–170.

Al-Saleh, I. A., Billed, G., & El-Doush, I. I. (2006). Levels of selenium, dl α-tocopherol, dl-γ- tocopherol, all-trans-retinol, thymoquinone, and thymol in different brands of *Nigella sativa* seeds. *Journal of Food Composition and Analysis, 19*(2–3), 167–175. https://doi.org/10.1016/j.jfca.2005.04.011

Bajpai, P., Usmani, S., Kumar, R., & Prakash, O. (2024). Recent advances in anticancer approach of traditional medicinal plants: A novel strategy for cancer chemotherapy. *Intelligent Pharmacy, 2*(3), 291–304. https://doi.org/10.1016/j.ipha.2024.02.001

Block, G., Dietrich, M., Norkus, E. P., Morrow, J. D., Hudes, M., Caan, B., & Packer, L. (2002). Factors associated with oxidative stress in human populations. *American Journal of Epidemiology, 156(3), 274–285.* https://doi.org/10.1093/aje/kwf029

Boztaş, G., Avcı, A. B., Arabacı, O., & Bayram, E. (2021). Economic status of medicinal and aromatic plants in the world and in Türkiye. *Theoretical and Applied Forestry, 1*, 27–33.

Cai, Y., Luo, Q., Sun, M., & Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences, 74*, 2157–2184. https://doi.org/10.1016/j.lfs.2003.09.047

Chaachouay, N., & Zidane, L. (2024). Plant-derived natural products: a source for drug discovery and development. *Drugs and Drug Candidates, 3*(1), 184–207. https://doi.org/10.3390/ddc3010011

Chank, X., Zhanq, T., Zhank, W., Zhao, Z., & Sun, J. (2020). Natural drugs as a treatment strategy for cardiovascular disease through the regulation of oxidative stress. *Oxidative Medicine and Cellular Longevity, 2020*, 5430407. https://doi.org/10.1155/2020/5430407

Chaudhry, G. E. S., Zeenia, Sharifi-Rad, J., & Calina, D. (2024). Hispidulin: a promising anticancer agent and mechanistic breakthrough for targeted cancer therapy. *Naunyn-Schmiedeberg's Archives of Pharmacology, 397*(4), 1919–1934. https://doi.org/10.1186/s12917-024-03983-3

Chehregani, A., Atri, M., Yousefi, S., Albooyeh, Z., & Mohsenzadeh, F. (2013). Essential oil variation in the populations of *Artemisia spicigera* from northwest of Iran: chemical composition and antibacterial activity. *Pharmaceutical Biology, 51*(2), 246–252. https://doi.org/10.3109/13880209.2012.717631

Chen, Y., Chang, H., Wang, C., & Cheng, F. (2009). Antioxidative activities of hydrolysates from duck egg white using enzymatic hydrolysis. *The Asian-Australasian Journal of Animal Sciences, 22*(11), 1587–1593.

Cuendet, M., Hostettmann, K., & Potterat, O. (1997). Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. *Helvetica Chimica Acta, 80*, 1144–1152.

Czechowski, T., Larson, T. R., Catania, T. M., Harvey, D., Wei, C., Essome, M., Brown, G. D., & Graham, I. A. (2018). Detailed phytochemical analysis of high-

and low artemisinin-producing chemotypes of *Artemisia annua*. *Frontiers in Plant Science, 9*, 641. https://doi.org/10.3389/fpls.2018.00641

Ekiert, H., Klimek-Szczykutowicz, M., Rzepiela, A., Klin, P., & Szopa, A. (2022). *Artemisia* species with high biological values as a potential source of medicinal and cosmetic raw materials. *Molecules, 27*(19), 6427. https://doi.org/10.3390/molecules27196427

Erel, O. (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clinical Biochemistry, 37*(4), 277–285. https://doi.org/10.1016/j.clinbiochem.2003.11.015

Gamez-Meza, N., Noriega-Rodriguez, J. A., Medina-Juarez, L. A., Ortega-Garcia, J., Cazarez-Casanova, R., & Angulo-Guerrero, O. (1999). Antioxidant activity in soybean oil of extracts from Thompson grape bagasse. *Journal of the American Oil Chemists' Society, 76*, 1445–1447.

Ghorbani, S., Kosari Nasab, M., Mahjouri, S., Talebpour, A. H., Movafeghi, A., & Maggi, F. (2021). Enhancement of *in vitro* production of volatile organic compounds by shoot differentiation in *Artemisia spicigera*. *Plants, 10*, 208. https://doi.org/10.3390/plants10020208

Golubkina, N., Shevchuk, O., Logvinenko, L., Molchanova, A., & Plugatar, Y. V. (2018). Macro and trace element accumulation by species of genus *Artemisia* on the southern coast of the Crimea. *Acta Horticulturae, 1324*, 389–394. https://doi.org/10.17660/ActaHortic.2021.1324.60

Gülçin, İ. (2025). Antioxidants: a comprehensive review. *Archives of Toxicology, 99*, 1893–1997. https://doi.org/10.1007/s00204-025-03997-2

Gülçin, İ., & Alwasel, S. H. (2023). DPPH radical scavenging assay. *Processes, 11*(8), 2248. https://doi.org/10.3390/pr11082248

Kafshboran, H. R., Dehghan, G., & Aghdam, M. N. (2011, January 7–9). Investigation of radical scavenging potential of some populations of Artemisia spicigera in relation to their flavonoid content. In *Proceedings of International Conference on Life Science and Technology.* Mumbai, India.

Ključevšek, T., & Kreft, S. (2025). Allergic potential of medicinal plants from the Asteraceae family. *Health Science Reports, 8*(2), e70398. https://doi.org/10.1002/hsr2.70398

Konovalov, D. A. (2014). Polyacetylene compounds of plants of the Asteraceae family. *Pharmaceutical Chemistry Journal, 48*, 9. https://doi.org/10.1007/s11094-014-1159-7

Kunchandy, E., & Rao, M. N. A. (1990). Oxygen radical scavenging activity of curcumin. *International Journal of Pharmaceutics, 58*(3), 237–240. https://doi.org/10.1016/0378-5173(90)90201-E

Lamaison, J. L., Carnat, A., & Petitjean, C. (1990). Tannin content and inhibiting activity of elastase in Rosaceae. *Annales Pharmaceutiques Françaises, 48*(6), 335–340.

Lee, J. H., Lee, J. M., Lee, S. H., Kim, Y. G., Lee, S., Kim, S. M., & Cha, S. W. (2015). Comparison of artemisinin content and antioxidant activity from various organs of *Artemisia* species. *Horticulture, Environment, and Biotechnology, 56*, 697–703. https://doi.org/10.1007/s13580-015-0143-9

Maridass, M., & De Britto, A. J. (2008). Origins of plant derived medicines. *Ethnobotanical Leaflets, 12*(1), 373–387.

Marinova, G., & Batchvarov, V. (2011). Evaluation of the methods for determination of the free radical scavenging activity by DPPH. *Bulgarian Journal of Agricultural Science, 17*(1), 11–24.

Öztürkmen, L. (1996). *Pharmacognostical studies on Artemisia spicigera C. Koch* (PhD thesis). IU Health Sciences Institute, İstanbul, Türkiye.

Pellegrini, N., Re, R., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9–10), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Peplow, M. (2018). Looking for cheaper routes to malaria medicines. *Chemical Engineering News, 96*(11), 29–31. http://doi/abs/10.1021/op4003196

Petretto, G. L., Chessa, M., Piana, A., Masia, M. D., Foddai, M., Mangano, G., Culeddu, N., Afifi, F. U., & Pintore, G. (2013). Chemical and biological study on the essential oil of *Artemisia caerulescens* L. ssp. *densiflora* (Viv.). *Natural Product Research, 27*, 1709–1715. https://doi.org/10.1080/14786419.2012.749471

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry, 269*, 337–341. https://doi.org/10.1006/abio.1999.4019

Rolnik, A., & Olas, B. (2021). The plants of the Asteraceae family as agents in the protection of human health. *International Journal of Molecular Sciences, 22*(6), 3009. https://doi.org/10.3390/ijms22063009

Ruch, R. J., Cheng, S. R., & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis, 10*(6), 1003–1008. https://doi.org/10.1093/carcin/10.6.1003

Sahin, A., Kıran, Y., Karataş, F., & Sönmez, S. (2005). Vitamins A, C, and E and β-Carotene content in seeds of seven species of *Vicia* L. *Journal of Integrative Plant Biology, 47*(4), 487–493. https://doi.org/10.1111/j.1744-7909.2005.00083.x

Sen, S., Chakraborty, R., Sridhar, C., Reddy, Y. S. R., & De, B. (2010). Free radicals, antioxidants, diseases and phytomedicines: Current status and future prospect. *International Journal of Pharmaceutical Sciences Review and Research, 3*(1), 91–100.

Soni, R., Singh, A., & Gupta, V. (2024). Efficient in vitro regeneration of *Artemisia annua*: Effect of different combinations of plant growth regulators on various types of explants. *Plant Biosystems, 158*(1), 59–69. https://doi.org/10.1080/11263504.2023.2287538

Tao, G., Dagher, F., Moballegh, A., & Ghose, R. (2020). Role of oxidative stress in the efficacy and toxicity of herbal supplements. *Current Opinion in Toxicology, 20–21*, 36–40. https://doi.org/10.1016/j.cotox.2020.04.004

Tumilaar, S. G., Hardianto, A., Dohi, H., & Kurnia, D. (2024). A comprehensive review of free radicals, oxidative stress, and antioxidants: Overview, clinical applications, global perspectives, future directions, and mechanisms of antioxidant activity of flavonoid compounds. *Journal of Chemistry, 2024*(1), 5594386. https://doi.org/10.1155/2024/5594386

Xiao, L., Tan, H., & Zhang, L. (2016). *Artemisia annua* glandular secretory trichomes: The biofactory of antimalarial agent artemisinin. *Science Bulletin, 61*(1), 26–36. https://doi.org/10.1007/s11434-015-0980-z

Yi, O. S., Meyer, A. S., & Frankel, E. N. (1997). Antioxidant activity of grape extracts in a lecithin liposome system. *Journal of the American Oil Chemists' Society, 74*(10), 1301–1307.

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64*, 555–559.

Zurera, G., Estrada, B., Rineon, F., & Pozo, R. (1987). Lead and cadmium contamination levels in edible vegetables. *Bulletin of Environmental Contamination and Toxicology, 38*, 805–812.