Essential Oil Content, Antioxidative Characteristics and Enzyme Inhibitory Activity of *Sideritis akmanii* Aytaç, Ekici & Dönmez

*Sideritis akmanii* Aytaç, Ekici & Dönmez Uçucu Yağ İçeriği, Antioksidatif Özellikleri ve Enzim İhibitör Aktivitesi

**Short Title:** Phytochemical properties of *Sideritis akmanii* Aytaç, Ekici&Dönmez

**Kısa Başlık:** Sideritis Akmanii Aytaç, Ekici & Dönmez’in Fitokimyasal Özellikleri

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26.03.2021
25.06.2021
05.07.2021

**ABSTRACT**

**Objectives:** Our research aims to measure the essential oil analysis mineral substance profiles, total phenolic substance content, free radical scavenger properties, antioxidant capacity, and enzyme inhibitory activity of *Sideritis akmanii* Aytaç, Ekici & Dönmez, 1996.

**Materials and Methods:** A mixture of *S. akmanii* plant roots and stems were used. Essential fatty acid components of *S. akmanii* were determined by Gas chromatography-mass spectrometry (GC-MS) and bioelement concentrations by Inductively coupled plasma optical emission spectrometry (ICP-OES). The antioxidant activity of extracts was screened by 2,2-diphenyl1-picrylhydrazyl (DPPH) radical scavenging activities, total phenolic content, total antioxidant status (TAS), total oxidant status (TOS) analysis. Cholinesterase (ChE), α-glucosidase, α-amylase, tyrosinase inhibitory activity determined.

**Results:** The results demonstrated that the phenolic substance content was higher in methanol extract (144.08 ± 2.01 μg GAE/mg extract). DPPH scavenging effect of *S. akmanii* methanol extract (73.2%) was higher than acetone extract (60.1%). TAS values of extract methanol and acetone were 2.32 ± 0.4 and 2.38 ± 0.2 μmol Trolox Eq/g, TOS values were 4.88 ± 0.6 and 5.04 ± 0.5 μmol H₂O₂ Eq/g, and OSI values were 2.1 ± 0.3 and 2.11±0.24 arbitrary units, respectively. Hexadecanoic acid (17.9%) was found as the main component in the plant essential oil. *S. akmanii* species was prominent with high Mg and Al concentrations. Anticholinesterase activity
was determined that acetone extract (42.95%±0.90; 217.37±0.81 mg GALAEs/g) exhibited higher than methanol (33.33%±1.81; 208.76±1.62 mg GALAE/g). α-amylase inhibition was high in methanol extract (53.62 ± 1.85mmol ACEs/g extract) compared to acetone (47.73 ± 0.92mmol ACEs/g extract). The tyrosinase inhibitory activity of S. akmanii was determined very low inhibition of the reference compound.

**Conclusion:** It has been determined that S akmanii Aytaç, Ekici & Dönmez extracts have antioxidant properties and can inhibit acetylcholineesterase, α-glucosidase, α-amylase enzymes. The present study is informative on future studies on S. akmanii a highly bioavailable species and very extensive studies should be carried out.

**Keywords:** Sideritis akmanii aytaç, ekici&dönmez, antioxidative activity, bioelement, essential oil, enzyme inhibitory activity.

**ÖZ**

**Amaç:** Sideritis akmanii Aytaç, Ekici ve Dönmez, 1996'nın antioksidan kapasitesini, toplam fenolik madde içeriğini, serbest radikal temizleyici özelliklerini, mineral madde profillerini, enzim inhibitör aktivitelerini ve uçucu yağ analizini belirlemektir.

**Gereç ve Yöntemler:** S.akmanii bitki kökleri ve saplarından oluşan bir karışım kullanıldı. Bitkinin temel yağ asidi bileşenleri GC-MS ve bioelement konsantrasyonları ICP-OES ile belirlendi. Metanol ve aseton özütünün antioksidan aktivitesi, DPPH radikali süpürücü etkisi, toplam fenolik içerik, toplam antioksidan durumu (TAS), toplam oksidan durumu (TOS) analizi ile tanırdı. Kolinesteraz (ChE), α-amilaz, α-glukozidaz, tirozinaz inhibitör aktivitesi belirlendi.

**Bulgular:** Sonuçlar, metanol ekstraktında fenolik madde içeriğinin daha yüksek olduğunu gösterdi (144.08 ± 2.01 μg GAE / mg ekstrakt). S. akmanii metanol ekstresinin radikal süpürücü etkisi (% 73,2) aseton ekstraktındaki (% 60,1) daha yüksekti. Metanol ve aseton ekstraktlarının sırasıyla TAS değerleri 2.32 ± 0.4 ve 2.38 ± 0.2 μmol Trolox Eq / g, TOS değerleri 4.88 ± 0.6 ve 5.04 ± 0.5 μmol H2O2 Eq / g ve OSI değerleri 2.1 ± 0.3 ve 2.11 ± 0.24 dir. Bitkisel uçcu yağda hekzadekanoil asit (% 17.9) ana bileşen olarak tespit edilmiştir. S. akmanii türleri yüksek Mg ve Al konsantrasyonları ile ön çıkmıştır. Antikolinesteraz aktivitesi, aseton ekstraktının (% 42.95 ± 0.90; 217.37 ± 0.81 mg GALAEs / g) metanolden (% 33.33 ± 1.81; 208.76 ± 1.62 mg GALAE / g) daha yüksek olduğu belirlenmiştir. a-amilaz inhibisyonu, asetonla (47.73 ± 0.92 mmol ACE / g özüt) karşılaştırıldığında metanol özütünde (53.62 ± 1.85 mmol ACE / g özüt) yüksekti. S. akmanii'nin tirozinaz inhibe edici aktivitesi, referans bileşiğe göre çok düşük inhibisyon gösterdiği belirlendi.

**Sonuç:** S akmanii Aytaç, Ekici & Dönmez özleri antioksidan özelliklerine sahip olduğu ve asetilkolineesteraz, α-glukozidaz, α-amilaz enzimlerini inhibe edebildiği tespit edilmiştir. Bu çalışma, biyoyararlanmanın yüksek bir tür olan S. akmanii ile ilgili gelecekte yapılacak çalışmalar hakkında bilgilendirici nitelikte olup, hücre kültürü ve hayvan deneylerini içeren çok kapsamlı çalışmaların yapılması gerekmektedir.

**Anahtar Kelimeler:** Sideritis akmanii aytaç, ekici & dönmez, antioksidatif aktivite, bioelement, uçcu yağ, enzim inhibitör aktivite
INTRODUCTION

*Sideritis akmanii* Aytaç, Ekici & Dönmez is an aromatic herbaceous plant with light green leaves and can grow up to 1 meter high. On the top, there are mucronate leaves and the leaves are 0.5 mm thick with a yellowish color. *Sideritis* species commonly known as mountain tea and clary has been used since ancient times in medicine due to their antibacterial, antifungal, antiviral, antiseptic, analgesic, antispasmodic, carminative, and antidiabetic properties. It is not exported and mostly utilized in the domestic market in Turkey. It is an endangered species due to its use in grazing and gathering in large quantities for animal feed production. Thus, the preservation and culturing of this economically significant species is necessary.¹

The enzyme inhibition theory is very popular in the treatment strategies for universal health problems such as diabetes and Alzheimer's. According to this theory, the key enzymes that play a role in the pathology of diseases are inhibited to alleviate symptoms caused by disease.² α-amylase and α-glucosidase enzymes are the main enzymes of sugar metabolism and their activity increases blood glucose level. In this sense, inhibition of these enzymes is an important mechanism in the control of blood glucose level in diabetes.³ Tyrosinase is an oxidase that is the rate-limiting enzyme for controlling the production of melanin and the inhibition of this enzyme is the main way of controlling skin diseases.⁴ Acetylcholine esterase catalyzes the breakdown of acetyl and choline in the synaptic space. Increased cognitive functions by inhibiting this enzyme in Alzheimer’s patients and this are called a cholinergic hypothesis.⁵

The present study aimed to determine the essential oil analysis mineral substance profiles, total phenolic substance content, free radical scavenger properties, antioxidant capacity, and enzyme inhibitory activity of methanol and acetone extracts of *Sideritis akmanii* Aytaç, Ekici & Dönmez, collected at Kumalar Mountain in Afyon province in Turkey.

MATERIALS and METHOD

*Plant material*

*S. akmanii* plant was collected at 1880 m altitude at Kumalar Mountain, Çakmaktepe Pass, Şuhut/Afyonkarahisar (37°07'19.245", UTM 4511056) in August 2015, and it was identified by Dr. xx. The samples of the plant locally known as “tail tea” are stored at xx University, Faculty of Sciences and Literature herbarium.

*Preparation of extracts*

A mixture of *S. akmanii* plant roots and stems were used. These parts were crushed into small pieces and dried at ambient temperature in the shade. To prepare the extracts, 20 g *S. akmanii* powder was mixed with 400 mL solvent.⁶ Extracts were used to determine free radical scavenging activity and total phenolic content. For the antioxidant status analysis, 1 g dried and
pulverized plant was sonicated after adding 10 mL solvent. The product was then filtered through paper and centrifuged. The obtained supernatant was centrifuged again for use in the analyses. To determine *S. akmanii* mineral substance content, 0.5 g dried and pulverized plant was placed in a microwave to decompose the organic components. Preparation of plant ethanol extracts for enzyme inhibition analysis was prepared at 100 μg/mL concentration. Acetone and methanol extracts were prepared by dissolving in ethanol for enzyme inhibition analyzes. 10 mg (0.01 g) of the extract was first dissolved in 1 mL of ethanol and a 10 mg/mL stock was prepared. 100 μg/mL extracts were prepared from the prepared stock material.

**Isolation of essential oil**

For the extraction of essential oils from *S. akmanii* by hydrodistillation using a Clevenger type apparatus in 2.5 h. At the end of the distillation, the essential oil was collected, dried under anhydrous Na₂SO₄ and stored at 4 °C in the dark until analysis.

**Determination of DPPH Radical Scavenging Activity**

The DPPH radical scavenging activities of methanol and acetone extracts from root and stem parts of *S. akmanii* species were determined with the Blois method. DPPH was used as free radical. Ethanol was added to samples at concentrations of 45, 90 and 135 μg/mL. Stock DPPH was added to the samples and allowed to incubate for 30 minutes in the dark and at room temperature. Absorbance measurements were recorded at 517 nm against ethanol blank.

**Determination of Total Phenolic Content**

Total phenolic content of the extracts was determined using the Folin-Ciocalteu method. Folin-Ciocalteu reagent was added to the plant extracts and standard antioxidant solutions. Samples were stored in room for 2 hours with the addition of Na₂CO₃. Absorbance measured against water at 760 nm. The graph was plotted using the Gallic acid standard. The total amount of phenolic compounds in both extracts was calculated as Gallic acid equivalent (GAE) using the formula obtained from the graph (r²: 0.9881). The standard Gallic acid graph developed for this purpose is presented in Figure 1.

**TAS, TOS and OSI Index**

Total antioxidant and total oxidant status of *S. akmanii* was measured using commercial kits (Rel Assay, Gaziantep, Turkey). The OSI index as an indicator of oxidative stress was calculated by dividing the total oxidant state by the total antioxidant state.

**Determination of the Mineral Substance Content**

A microwave oven (Speed Wave ERGHOF) was used to deform the organic compounds in the samples. ICP-OES (Spectro Genesis) bioelement concentrations were measured after the samples were ready for analysis.

**GC and GC/MS analysis**
Volatile fatty acid components in the 1 ml sample injected into the system were determined on the GC-MS device. The analysis conditions of the device are given in Table 1.

**Table 1. GC-MS Analysis Conditions**

**Enzyme inhibitory activity**

Ellman method was used to determine the Cholinesterase (ChE) inhibitory activity of the extracts and in a 96-well microplate using eliza reader.\(^1\) Sample solution (100 μg/mL, 50 μL) was mixed with DTNB (0.3 mM, 125 μL) and AChE solution (0.026 U/ml, 25 μL) in Tris–HCl buffer (pH 8.0) in a 96-well microplate and incubated for 15 min at 25 °C. The reaction was initiated by the addition of 1.5 mM 25 μL of acetyl thio quinoline iodide (ATCI) substrate. Similarly, enzyme-free tube was prepared as a blank. Control sample for % inhibition calculation was prepared by the addition of ethanol instead of the sample. Sample, control, and blank absorbances were read at 405 nm after incubation at 25 °C for 10 minutes. Galantamine was used as standard. Standard sample solutions of 0.5-0.0005 μmol/mL were prepared and the calibration graph is plotted. Results were expressed both equivalents of galanthamine (mg GALAEs/g extract) and % inhibition value. Equality is given below to calculate the percent inhibition value.

\[
\% I = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \\
A_{control}: \text{Absorption obtained by adding solvent used instead of plant extract} \\
A_{sample}: \text{absorbance of plant extract}
\]

α-amylase inhibitor activity was performed using Caraway-Somogy iodide/potassium iodide (IKI) method.\(^2\) Sample solution at 100 μg/ml concentration (25 μL) was mixed with α-amylase solution (50 μL) in phosphate buffer (pH 6.9 with 6 mM sodium chloride) in a 96-well microplate and incubated for 10 min at 37 C. After the pre-incubation, the reaction was started by adding 50 μL of 0.05% starch solution. Similarly, the enzyme-free blank solution and control samples containing solvent were prepared instead of samples. The reaction was stopped by the addition of HCl (25 μL, 1 M) and iodine-potassium iodide solution (100 μL) was added. Acarbose was used as standard material.

Standard sample solutions of 2 μmol/ml, 1 μmol/ml, 0.1 μmol/ml, 0.01 μmol/ml and 0.001 μmol/ml were prepared and a calibration graph was drawn. The results of α-amylase inhibitor activity were expressed as equivalent to both acarbose equivalent (mmol AKAE / g) and the % inhibition value used for calculation.

α-glucosidase inhibitor activity was performed according to the method.\(^3\) Sample solution (100 μg/mL ekstrakt, 50μL) was mixed with glutathione (2 mg/mL,50 μL), α-glucosidase solution (2 U/ml, 50 μL) in phosphate buffer (pH 6.8) and 4-Nitrofenil β-D-glukuronid (PNPG) (10mM, 50 μL) in a 96-well microplate. Similarly, enzyme-free blank solution and control samples containing solvent instead of sample were prepared. The reaction was stopped by adding 50 μL of 0.2 M Na₂CO₃ after incubated for 15 min at 37 C°. Results was expressed to both acarbose (mmol AKAE / g) and the% inhibition value.
Dopacrome method modified with L-DOPA as substrate was used for determination of tyrosinase inhibitory activity. Extracts were dissolved in 50% DMSO and prepared at a concentration of 100 µg/mL. 40 µL of the sample solution, 20 µL of 480 U/mL tyrosinase enzyme solution and 120 µL of phosphate buffer (20mM pH 6.8 sodium phosphate buffer) were mixed in a 96-well microplate and incubated at 25 °C for 15 minutes. The reaction was initiated by addition of 20 µL of 2.5 mM L-DOPA. Control sample for % inhibition calculation prepared by the addition of ethanol instead of the sample. Samples and blank absorbances were read at 492 nm. Results are expressed as equivalent to both kojic acid (mgKAE/g) and % inhibition value.

Statistical Analyses

The mean of four separate analysis results were presented as analysis results. The statistical analysis was performed with the software SPSS 15.0 (SPSS Inc.) and findings were recorded as mean ± standard deviation.

RESULTS and DISCUSSION

S. akmanii is a perennial species that grows 1520-1550 m high steppes. The species flowers in July and August. It is collected to Kumalar Mountains in Afyonkarahisar province. S. akmanii species is in the endangered (EN) species category due to overgrazing and consumption as herbal tea.

Total phenolic substance content

Phenolic compounds were shown to have anti-oxidative, free radical scavenging, enzymatic activity regulating, cell proliferation inhibitive, anti-inflammatory properties in previous studies. Sideritis species are also a rich source of flavonoids. Major flavonoids found in Sideritis species were flavones, methoxyflavones, sideroflavones, cirsiliol and xanthomycin. Phytochemical studies on S. akmanii species reported sideridiol, sideroxol, linearol, isolinearol, folanol and isofiolol content.

The total phenolic content in the plants used in the study is presented in Table 2. As observed in Table 2, it was determined that S. akmanii plant methanol and acetone extract phenolic compound content was 144.08±2.01 and 117.72±6.4 µg GAE. It was observed that the phenolic compounds in the plant and the solvents used in the determination of antioxidant activity led to different results. It was found that the plant acetone extract contained higher phenolic compound levels when compared to the methanol extract. This finding also provides information on higher antioxidant capacity due to the higher phenolic content of the species due to the procedures that would be conducted with an adequate solvent.

Table 2 Total phenolic compound content in 1 mg S. akmanii methanol and acetone extracts

DPPH radical scavenging activity
The DPPH method is often used to determine radical scavenging activities of plants. There are also studies conducted with *Sideritis* species in the literature. A comprehensive study that all *Sideritis* species had high antioxidant and DPPH radical scavenging properties due to their high phenolic substance content.\textsuperscript{18} *Sideritis caesarea*, which has a higher total phenolic substance and flavonol content, had DPPH radical scavenging properties.\textsuperscript{19} In previous studies, it was determined that the high radical scavenging effect of methanol extracts was associated with the total amount of phenolic substance content in methanol extracts. Similarly, in the present study, it was found that the radical scavenging properties of this extract were higher due to its high phenolic content.

DPPH radical scavenging activities of *S. akmanii* methanol and acetone extracts are presented in Figure 2. It was observed that the DPPH radical scavenging activities of the extracts increased with the concentration. The DPPH radical scavenging activities of *S. akmanii* methanol and acetone extracts and the synthetic antioxidant BHT and 135 μg/ml concentration of the natural antioxidant α-tocopherol were determined as follows: α-tocopherol > *S. akmanii* methanol extract (SAM) ~ BHT > *S. akmanii* acetone extract (SAA). The DPPH radical scavenging activities of *S. akmanii* methanol and acetone extracts were 78.7% in α-tocopherol, 73.2% in methanol extract, 72.7% in BHT and 60.1% in acetone extract. Results suggested that the *Sideritis akmanii* methanol extract had similar activities with BHT, a synthetic antioxidant.

**TAS, TOS and OSI values**

There are several methods to determine the total antioxidant activities in plant extracts or active substances isolated from plants.\textsuperscript{20} Solitary measurement of antioxidants requires time consuming, expensive and complex techniques. For this reason, total antioxidant capacity or total antioxidant status measurement is the currently preferred and widely used methodology. TAS; TOS and OSI values for *S. akmanii* plant methanol and acetone extracts are presented in Figure 3. It was determined that *S. akmanii* plant methanol and acetone extract TAS values were 2.32 ± 0.4 and 2.38 ± 0.2 μmol Trolox Eq/g, TOS values were 4.88 ± 0.6 and 5.04 ± 0.5 μmol H$_2$O$_2$ Eq/g, and OSI values were 2.1 ± 0.3 and 2.11±0.24 arbitrary units, respectively. These results demonstrated that the TAS values were similar in both plant extracts. The acetone extract TOS level was higher when compared to the methanol extract, however it was found that the oxidative stress index, which is an indicator of the oxidative stress, was similar for both extracts.

**Sideritis akmanii species mineral substance content**

In a study that aimed to determine the bio-element content in endemic *Sideritis* species (*Sideritis germanico politana, Sideritis galatica*, and *Sideritis Hispana*), it was determined that the heavy metal concentrations in *Sideritis* species were within the limits for human consumption without health risks and the *Sideritis* species indigenous to Turkey could be considered as a significant source for certain nutritional elements such as iron and potassium.\textsuperscript{21}

In the present study, the mineral substance profile of *S. akmanii* species was also examined. As is the case with other *Sideritis* species, *S. akmanii* species was prominent with high Mg and Al concentrations, in addition to Fe and K content. Furthermore, Zn, Mn and Cu concentrations in the antioxidant enzyme structure were also very high. However, since the concentration of Se element incorporated in the glutathione peroxidase structure was not within
the measurable levels, it could not be recorded. Table 2 summarizes the mineral substance levels in *S. akmanii* species.

Table 3 Mineral substance and concentrations of *S. akmanii*

As shown in Table 3, Se, Be, and Co element concentrations were not within the detectable limits, so concentrations for these elements could not be determined. It was determined that all other minerals, which were identified as antioxidants, were observed at sufficient levels *S. akmanii* species, except Se.

**GC-MS Analysis Results**

*S. akmanii* plant collected from Afyonkarahisar province dry oil yield was determined as 0.13%. Palmitic acid (17.9%), Myristic acid (11.0%) and Spatulenol (8.0%) were found as the main components in the plant essential oil (Table 4).

Table 4 Essential Oil Composition of *S. akmanii*

The composition of *Sideritis* species volatile oil divided into 6 groups; rich in monoterpenes, rich in oxygenated monoterpenes, rich in sesquiterpenes, rich in oxygenated sesquiterpenes, rich in diterpenes and others. In addition, high oil yield of plants obtained from the essential oil composition monoterpenes rich, plants with low fat yield were found to be rich in sesquiterpene compounds. Studies conducted with *Sideritis* genus demonstrated that the plants contain terpenes, flavonoids, iridoids, kumarin, lignan and sterols. Diterpen and flavonoid structures are found in almost all species. It was considered that the pharmacological activity of the species originate from these structures. While sesquiterpen and triterpen structures are not common in *Sideritis* species, diterpene structures are quite common. About 160 different diterpene species were found in these species. Especially in Mediterranean countries (Turkey, Greece and Italy), it was observed that *Sideritis* species contained kauri diterpenes. Kauren diterpene derivatives, namely foliol, cidol, linearol, sideridiol and isolinearol were identified in the species.

In our study, the presence of pinene component in *S. akmanii* volatile oil was not detected and the presence of sesquiterpenes such as β-caryophylen (1.1%) caryophyllen oxide (5.7%) α-Bisabolol (1.5%) were determined. More ent-kauren diterpenes are obtained from the plants grown in the Eastern Mediterranean region. As a result of this study, only kaur-15-en diterpen was obtained.

**Enzyme Inhibitory Activities**

**Anticholinesterase activity**

Alzheimer's disease (AD) is an age-related neurodegenerative disorder and the most common form of dementia in the elderly. AChE inhibitors are used in the treatment of Alzheimer's disease and many researchers have attempted to identify novel AChE inhibitors from plant sources. In this study, the anticholinesterase activity of *S. akmanii* was investigated for
the first time. It was determined that acetone extract (42.95 ± 0.90; 217.37 ± 0.81 mg GALAEs/g) exhibited higher anticholinesterase activity than methanol (33.33 ± 1.81; 208.76 ± 1.62 mg GALAE/g) (Table 5). It has been reported that essential oil components such as oxygenated monoterpenes compounds are the main agents responsible for the effect of anti-Alzheimer.29 According to the GC-MS results, the fatty acid composition of S. akmanii contains terpene compounds such as β-caryophyllene and α-Bisabolol. It has been reported that phenolic acids such as ferulic acid and p-coumaric acid exhibits strong anticholinesterase activity in extracts.31 Diterpenes isolated from Sideritis arguta showed no inhibition activity against acetylcholinesterase enzyme.32 When our results are evaluated, AChE inhibitory activity of S. akmani extracts perform quite well when compared to the standard used galantamine. The higher activity of acetone extract than methanol reveals how the solvent used can alter the enzyme activity.

Table 5 AChE Enzyme Inhibition Results of S. akmanii Methanol and Acetone Extract

**Antidiabetic activity**

One of the therapeutic approaches for reducing blood sugar is to delay glucose absorption by inhibition of carbohydrate hydrolysis enzymes such as α-amylase and α-glucosidase in digestive organs.33 Therefore, studies to develop new pharmacological agents are related to α-amylase and α-glucosidase inhibition.5,34 It has been reported that some natural extract components containing phenolics, flavonoids and their glycosides exhibit α-glucosidase inhibitory effect. It has long been known that polyphenolic compounds in plants inhibit the activity of digestive enzymes due to their ability to bind proteins.35 Since these molecules have antioxidant effects, it is possible that the antioxidant and α-glucosidase inhibitory properties are due to their polyphenolic content.

In this study, α-amylase inhibition was high in methanol extract (53.62 ± 1.85 mmol ACEs/g extract) compared to acetone (47.73 ± 0.92 mmol ACEs/g extract). In addition, when we examined the percent inhibition, it was found that the results were very close to the acarbose and the results of the methanol and acetone extracts were close to each other. α-amylase inhibition value of the methanol extract of Sideritis galatica, 0.41 ± 0.01 mmol ACEs/g, and the α-glucosidase inhibition value was 1.68 ± 0.28 mmol ACEs/g. At the same time, Sideritis galactica petroleum ether and ethyl acetate extracts had higher α-amylase and α-glucosidase inhibition than methanol and water extracts.13 The relatively low inhibitory potential of Sideritis perfoliata extracts prepared with increasing polarity.36

Table 6 α-Amylase and α-Glucosidase Enzyme Inhibition Results of S. akmanii Methanol and Acetone Extract

The inhibition of amylase and glucosidase enzymes close to the acarbose standard may indicate that the plant has significant potential for the treatment of Type 2 diabetes. In conclusion, it can be concluded that the species S. akmanii, which has antioxidant properties, may be a promising pharmacological agent for the treatment of diabetes.

**Tyrosinase inhibitory activity**
Tyrosinase contains copper and is involved in melanin biosynthesis. It is also an enzyme that catalyzes the oxidation of tyrosine to DOPA and DOPA quinone. Melanin plays an important role in protecting the skin from ionizing radiation such as UV. Tyrosinase has been researched for its use in the food industry along with cosmetics and agriculture for many years. In recent years, its applications in the medical industry have gained considerable popularity because it has the effect of protecting from pigmentation and other skin disorders such as vitiligo, malignant melanoma. Common tyrosinase inhibitors, such as hydroquinone (HQ), kojic acid and arbutin, have some adverse effects on human health.

In this study, the tyrosinase inhibitory activity of *S. akmanii* was determined in vitro. Methanol (4.24% ± 0.146) and acetone extracts (8.07% ± 0.29) were observed to exhibit very low inhibition of the reference compound, α-kojic acid (86.34% ± 1.46) (Table 7). The antityrosinase effect of the methanol extract was 80.42 ± 0.95 mg KAE/g as the equivalent of the kojic acid, acetone extract was found as 105.42 ± 1.91 mg KAE/g extracts. Our study is important because it is the first study on the tyrosinase enzyme inhibiting activity of *S. akmanii*. Different solvent extracts and different concentrations of the plant should be studied to determine the tyrosinase enzyme inhibition.

Table 7 Tyrosinase Enzyme Inhibition Results of *S. akmanii* Methanol and Acetone Extract

**CONCLUSIONS**

In conclusion, it can be suggested that *Sideritis akmanii* Aytaç, Ekici & Dönmez methanol and acetone extracts possess antioxidant effects due to their phenolic substance content. The determined TAS, TOS and OSI values also support this suggestion. It was found that the radical scavenging activities in the methanol extract was similar to that of the synthetic antioxidant BHT and higher than that of the acetone extract. These findings indicated that the species, especially the methanol extract had antiradical effects. The mineral content of the species included important minerals (Mn, Zn, Fe, Cu) within the antioxidant enzyme structure. Due to the adverse effects of synthetic antioxidants, studies on natural antioxidant sources have increased during recent years. This is the first study to investigate the chemical composition and enzyme inhibitory potential of *S. akmanii*. As a result, methanol and acetone extracts of *S. akmanii* have enzyme inhibition activity and the data obtained will contribute to the search for new alternative drugs to be used in the treatment of diseases such as diabetes, Alzheimer's and Parkinson's. Identification of antioxidant activities and active components in plants, identification and purification of their structures constitute the first step in these studies. Due to their total phenolic content, their effect against the radicals, TAS, TOS, OSI values and their mineral content, it is considered that *S. akmanii* could be used in phytotherapeutical studies and particularly in studies on antioxidant effect.
ACKNOWLEDGEMENT

The present study was supported by the Scientific Research Projects Commission of the xx University, with the ID numbers 18.KARIYER.79 and 15.FEN.BIL.41

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
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Figures

Figure 1 Gallic acid standard curves
Figure 2 DPPH radical scavenging activities of plant extracts in different concentrations (45-135 µg/mL), SAM: *Sideritis akmanii* methanol extract SAA: *Sideritis akmanii* acetone extract BHT: Butylated hydroxytoluene and α-tocopherol.
Figure 3 TAS, TOS and OSI data for *Sideritis akmanii*. The results are presented as mean ± SD (n = 4). SAM: *Sideritis akmanii* methanol extract SAA: *Sideritis akmanii* acetone extract TAS: Total antioxidant status TOS: Total oxidant status OSI: Oxidative stress index
### Table 1. GC-MS Analysis Conditions

| Features                  | Description                                                                                                                                 |
|---------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| System                    | Agilent 7890 B GC5977B Mass Selective Detector system                                                                                       |
| Column                    | Agilent HP-Innowax (60 m, 0.25 mm inner diameter, 0.25 m film thickness)                                                                        |
| Injection temperature     | 250 °C                                                                                                                                        |
| Ion source temperature    | 230 °C                                                                                                                                        |
| Ionization Mode Hand      | EI                                                                                                                                           |
| Electron energy           | 70 eV                                                                                                                                       |
| Mass range                | 35-450 m/z                                                                                                                                   |
| The temperature program   | 60 °C (10 min), 4 °C/min. 220 °C (10 min) 1 °C/min. 240 °C (20 min.)                                                                           |
| Carrier gas               | Helium (0.7 mL/min)                                                                                                                        |
| Definitions Spectral      | Wiley 9-Nist 11 Mass                                                                                                                        |
| Database                  |                                                                                                                                              |

### Table 2. Total phenolic compound content in 1 mg *S. akmanii* methanol and acetone extracts

| Extract                                           | SAM               | SAA       |
|---------------------------------------------------|-------------------|-----------|
| Total Phenolic Compound Content (µg GAE/mg extre)  | 144.08±2.01       | 117.72±6.4|

The results are presented as mean±SD (n = 4). SAM: *S. akmanii* methanol extract; SAA: *S. akmanii* acetone extract
### Table 3 Mineral substance and concentrations of *S. akmanii*

| Mineral substance | Concentration (ppm) | Mineral substance | Concentration (ppm) |
|-------------------|---------------------|-------------------|---------------------|
| Al                | 891.64±46.78        | K                 | 1904.69±86.48       |
| B                 | 7.884±0.96          | Li                | 0.619±0.28          |
| Ba                | 31.02±0.98          | Mg                | 907.26±35.96        |
| Bi                | 3.634±0.64          | Mn                | 32.05±3.46          |
| Ca                | 1344.5±92.5         | Na                | 461.68±28.59        |
| Cr                | 2.13±0.17           | Ni                | 1.23±0.16           |
| Cu                | 6.23±1.82           | Pb                | 3.21±0.83           |
| Fe                | 1264.37±26.75       | Zn                | 10.01±2.06          |

The results are presented as mean ± SD (n = 4)
Table 4 Essential Oil Composition of *S.akmanii*

| No | Compound                                           | Relative percentage (%) |
|----|----------------------------------------------------|-------------------------|
| 1. | β-Burbone                                          | 0.5                     |
| 2. | β-Caryophyllen                                     | 1.1                     |
| 3. | α-Acoradiene                                       | 1.0                     |
| 4. | Curcumen                                           | 4.5                     |
| 5. | Caryophyllen oxide                                | 5.7                     |
| 6. | Hexahydrofarnesyl acetone                         | 1.5                     |
| 7. | Spatulenol                                         | 8.0                     |
| 8. | Turmerol                                           | 1.3                     |
| 9. | α-Bisabolol                                        | 1.5                     |
| 10. | 9-Geranyl-p-cymene                                 | 3.1                     |
| 11. | Kaur-15-en                                         | 0.6                     |
| 12. | Caryophylla-2(12),6-dien-5β-ol                    | 1.3                     |
| 13. | Manoyl oxide                                       | 1.3                     |
| 14. | Dodecanoic acid (Lauric acid)                      | 0.5                     |
| 15. | (E)-9-Octadecen-1-ol (Trans-Elaidyl Alcohol)      | 0.7                     |
| 16. | Tetradecanoic acid (Myristic acid)                 | 11.0                    |
| 17. | 8,12-Epoxy-13-hydroxy-14-ene-labdien               | 1.1                     |
| 18. | Pentadecanoic acid                                | 0.6                     |
| 19. | Hexadecanoic acid (Palmitic acid)                  | 17.9                    |
| 20. | (Z)-Hexadecenoic acid (Palmitoleic acid)          | 1.2                     |
| 21. | (Z)-9-Octadecenoik acid (Oleic acid)              | 2.2                     |
Table 5  Acetyl Choline Esterase Enzyme Inhibition Results of *S.akmanii* Methanol and Acetone Extract

| Extract                | AChE inhibition (mg GALAEs/g extract) | AChE inhibition (%) |
|------------------------|--------------------------------------|---------------------|
| SAM                    | 208.76± 1.62                          | 33.33±1.81          |
| SAA                    | 217.37±0.81                           | 42.95±0.90          |
| Galantamine (0.5 µmol/mL) |                                     | 51.28±0.01          |

Values expressed are means ± SD of four parallel measurements. GALAEs: galantamine equivalents; AChE: Acetyl Choline Esterase; SAM: *S. akmanii* methanol extract SAA: *S.akmanii* acetone extract
### Table 6 α-Amylase and α-Glucosidase Enzyme Inhibition Results of *S. akmanii* Methanol and Acetone Extract

| Extract       | α-Amylase Inhibition (mmol ACAEs/g extract) | α-Amylase Inhibition (%) | α-Glucosidase Inhibition (mmol ACAEs/g extract) | α-Glucosidase Inhibition (%) |
|---------------|---------------------------------------------|--------------------------|-----------------------------------------------|-----------------------------|
| SAM           | 53.62±1.85                                  | 45.39±0.22               | 59.23±0.49                                   | 76.82±0.09                  |
| SAA           | 47.73±0.92                                  | 46.11±0.11               | 62.37±0.98                                   | 76.25±0.18                  |
| Acarbose(1µmol/mL) | 51.36±0.12                           |                          |                                               | 85.81±2.45                  |

Results expressed are means ± SD of four parallel measurements. ACAE: Acarbose equivalent; SAM: *S. akmanii* methanol extract; SAA: *S. akmanii* acetone extract
| Extract                  | Tyrosinase Inhibition (mg KAEs/g ekstre) | Tyrosinase Inhibition (%) |
|--------------------------|------------------------------------------|---------------------------|
| SAM                      | 80.42±0.95                               | 4.24±0.146                |
| SAA                      | 105.42±1.91                              | 8.07±0.29                 |
| Kojic acid (2 µmol/ml)   |                                          | 86.34±1.46                |

Values expressed are means ± SD of four parallel measurements. KAEs: Kojic acid equivalent; SAM: *Sideritis akmanii* methanol extract SAA: *Sideritis akmanii* acetone extract