KSÜ Tarım ve Doğa Derg 25 (6): 1216-1224, 2022
KSU J. Agric Nat 25 (6): 1216-1224, 2022
https://doi.org/10.18016/ksutarimdoga.vi.997780

# Biological Activities of the Methanol Extracts of *Smyrnium connatum* Boiss. and Kotschy.

Fatma AYAZ[1], Nuraniye ERUYGUR[2], Tuğsen DOĞRU[3], Yavuz BAĞCI[4], Süleyman DOĞU[5]
[1,2,3]Department of Pharmacognosy, Faculty of Pharmacy, Selcuk University, Konya 42250, Türkiye, [4]Department of Pharmaceutical Botany, Faculty of Pharmacy, Selcuk University, Konya 42250, Türkiye, [5]Department of Animal and Plant Production, Meram Vocational School, Necmettin Erbakan University, Konya 42090, Türkiye
[1]https://orcid.org/0000-0003-3994-6576, [2]https://orcid.org/0000-0002-4674-7009, [3]https://orcid.org/0000-0003-0101-9742
[4]https://orcid.org/0000-0002-2343-3672, [5]https://orcid.org/0000-0002-5352-9288
✉: fatma.ayaz@selcuk.edu.tr

## ABSTRACT

*Smyrnium connatum* Boiss. and Kotschy is known as "yabani kereviz" in Turkey and its roots are used to treat asthma. *Smyrnium* species are used by people in different regions as diuretic, depurative and laxative. The aim of this analysis was to test the biological activities of methanol extracts obtained from aerial parts and roots of *S. connatum* using a Soxhlet extractor, ultrasonic assisted extraction, and maceration procedures. The extracts of *S. connatum* aerial parts and roots were tested for their acetylcholinesterase, butrylcholinesterase, and tyrosinase inhibitory activities, radical scavenging activity, and iron chelating activities at various concentration using microplate reader. In this study, significant differences were found in acetylcholinesterase, and butrylcholinesterase inhibition and antioxidant determinations between plant extracts prepared using different extraction methods. *S. connatum* aerial parts generally was detected to be more active in terms of antioxidant activity assay. *S. connatum* aerial parts and roots showed high inhibition activity against both cholinesterase enzymes. All extracts showed moderate inhibitory activity against tyrosinase. The biological activity of the aerial parts of *S. connatum* was generally found to be more active than roots of *S. connatum.* It was assumed that the determination of enzyme inhibitor and antioxidant capacity of the plant had remarkable potentials on the treatment of neurodegenerative diseases.

**Biochemistry**

**Research Article**

**Article History**
Received : 24.09.2021
Accepted : 20.12.2021

**Keywords**
*Smynrium connatum*
Enzyme inhibition
Antioxidant activity
Extraction

## *Smyrnium connatum* Boiss. and Kotschy. Metanol Ekstresinin Biyolojik Aktivitesi

## ÖZET

*Smyrnium connatum* Boiss. and Kotschy bitkisi Türkiye'de "yabani kereviz" olarak bilinir ve bitkinin kökleri astım tedavisinde kullanılır. Farklı bölgelerde *Smyrnium* türleri insanlar tarafından diüretik, depuratif ve laksatif amaçlarla kullanılmaktadır. Bu çalışmanın amacı, Soxhlet ekstraktörü, ultrasonik destekli ekstraksiyon ve maserasyon yöntemleri kullanılarak *S. connatum* toprak üstü ve köklerinden hazırlanan metanol ekstrelerinin biyolojik aktivitelerini değerlendirmektir. *S. connatum* toprak üstü ve kök ekstreleri, çeşitli konsantrasyonlarda asetilkolinesteraz, bütirilkolinesteraz ve tirozinaz inhibitör aktiviteleri, radikal temizleyici potansiyelleri ve demir şelatlama kapasiteleri bakımından mikroplaka okuyucu kullanılarak test edildi. Yapılan araştırmalarda, farklı ekstraksiyon yöntemleri kullanılarak hazırlanan bitki ekstreleri arasında hem enzim inhibisyonu hem de antioksidan aktiviteleri önemli ölçüde farklı bulunmuştur. *S. connatum* toprak üstü kısımlarının antioksidan aktivitesi bakımından genel olarak daha yüksek tespit edilmiştir. *S. connatum* toprak üstü kısımları ve kökleri, her iki kolinesteraza karşı da yüksek inhibitör aktivite göstermiştir. Tüm ekstreler, tirozinaza karşı orta düzeyde inhibitör aktivite göstermiştir. Genel olarak *S.*

**Biyokimya**

**Araştırma Makalesi**

**Makale Tarihçesi**
Geliş Tarihi : 24.09.2021
Kabul Tarihi : 20.12.2021

**Anahtar Kelimeler**
*Smynrium connatum*
Enzim inhibisyonu
Antioksidan aktivite
Ekstraksiyon

*connatum*'un toprak üstü kısımlarının biyolojik aktivitesi köklerine göre daha aktif bulundu. Enzim inhibitörü ve antioksidan kapasite tayinlerinin sonucunda bitkinin nörodejeneratif hastalıkların tedavisinde kayda değer sonuçlar verebilecek potansiyellere sahip olduğu düşünülmektedir.

**Atıf Şekli**: Ayaz F, Eruygur N, Doğru T, Bağcı Y, Doğu S 2022. *Smyrnium connatum* Boiss. and Kotschy. Metanol Ekstresinin Biyolojik Aktivitesi. KSÜ Tarım ve Doğa Derg 25 (6): 1216-1224. https://doi.org/10.18016/ksutarimdoga.vi.997780

**To Cite :** Ayaz F, Eruygur N, Doğru T, Bağcı Y, Doğu S 2022. Biological Activities of The Methanol Extracts of *Smyrnium connatum* Boiss. and Kotschy. KSU J. Agric Nat 25 (6): 1216-1224. https://doi.org/10.18016/ksutarimdoga.vi.997780

## INTRODUCTION

*Smyrnium* L., which comprises 38 species worldwide, contains six taxa in the Turkish flora; *S. olusatrum* L., *S. perfoliatum* L. subsp. *perfoliatum*, *S. perfoliatum* subsp. *rotundifolium* (Miller) Hartwig, *S. cordifolium* Boiss., *S. connatum* Boiss. and Kotschy and *S. creticum* Miller. (Stevens, 1972; Mungan et al., 2008; Mungan et al., 2011; Abbasi et al., 2019). *Smyrnium* taxa is widely used for the treatment of various diseases by people in different regions such as diuretic, depurative, and aperient activities, especially through their roots. However, because of the high vitamin C concentration, they may have the best antiscorbutic properties (Caprioli et al., 2014). Carminative and stomachic effects are present in the fruit (Bermejo and León, 1994; Minareci and Kalyoncu, 2012). Aerial parts, roots, and seeds of *S. cordifolium* have traditionally been used for the problems on urinary tracts and prostate, gynecological area, stomachic, and indigestion according to the ethnobotanical data, as well as for its properties, such as aromatic bitterness, heated effects, tonic, antihelmintic, antipyretic, antiworm in Iran (Amiri and Joharchi, 2016). In Manisa Turgutlu, it was reported that the decoction of *S. olusatrum* roots were used for abortion (Bulut and Tuzlaci, 2013). In an ethnobotanical study conducted in Andırın, Kahramanmaraş, it was reported that the fresh roots of *S. connatum* were used for the treatment of asthma (Demirci et al., 2014).

*S. connatum* is biennials, their stems are sturdy and ridged, measuring 70-150 cm in length. Ultimate segments are oval, at least 2x1.5 cm, bluntly serrate; basal leaves are 3-pinnate/ternate. Upper stem leaves are opposite, with at least some connate, oblong, up to 10 cm long, and subentire to obscurely serrulate. Umbels 10-17 have been rayed. Style is 0.9-1.3 mm, reflexed; mericarps are 2.8x2.3 mm, dorsal ridges are indistinct (Davis, 1972).

Overproduction of oxidants (reactive oxygen species and reactive nitrogen species) in the human body have serious adverse effects in the pathogenesis of many chronic diseases such as cardiovascular diseases, diabetes, obesity, neurological disorders (Alzheimer's, and Parkinson's disease etc.), and skin diseases. Therefore, protective role of phytochemicals such as polyphenolics, triterpenes, saponins, and alkaloids could be linked to their antioxidant activity (Zhang et al., 2015).

Alzheimer's disease is a progressive neurodegenerative condition marked by cholinergic system impairments and beta amyloid deposition in the form of neurofibrillary tangles and plaques. As a result, inhibition of acetylcholinesterase (AChE) and butyrlcholinesterase (BuChE) has been identified as a significant target for the effective management of Alzheimer's disease, resulting in an increase in acetylcholine availability in brain areas and a decrease in amiloid plaque development (Anand and Singh, 2013; Alam and Sharma, 2019).

Melanogenesis is the process of synthesizing melanin, which is the pigment that gives color to human skin, eyes, and hair. Tyrosinase (TYR) is a crucial enzyme that catalyzes a rate-limiting step in the production of melanin. As a result, many TYR inhibitors have been developed for skin whitening in recent years (Pillaiyar et al., 2017).

The aim of this study is to evaluate the cholinesterase and TYR inhibition and antioxidant activities of the extracts obtained from *S. connatum* aerial parts (ASC) and roots (RSC) and to compare the effects of the extracts prepared by different extraction methods on the activity. Moreover, purpose was the determination of the antioxidant properties of the extracts using iron-chelating activities, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis-3 ethylbenzothiozoline-6-sulfonic acid (ABTS) cation decolorization test.

## MATERIALS and METHODS

### Plant material

*S. connatum* aerial parts and roots were collected from Adana, Turkey. The plant material was identified by Prof. Dr. Yavuz Bağcı. The voucher specimen was kept at the Herbarium of Selçuk University in Konya (KNYA) Turkey.

### Extraction methods

The methanol extracts prepared from *S. connatum*

aerial parts (ASC) and roots (RSC) with soxhlet extractor, ultrasound assisted and maceration methods.

Maceration: The aerial parts and roots of *S. connatum* were separately macerated in 70% MeOH at room temperature for one day, three times and then filtered. The filtrate was evaporated at 40 °C temperature with rotary evaporator (Bennour et al., 2020).

Ultrasound assisted: The aerial parts and roots of *S. connatum* were separately extracted in 70% MeOH using ultrasonic cleaner for 20 minutes with four times. After filtration, the filtrate was evaporated at 40 °C temperature with rotary evaporator.

Soxhlet extractor: The aerial parts and roots of *S. connatum* were separately extracted using soxhlet apparatus with 70% methanol for 6 h and then filtered. Methanol was removed using a rotary evaporator.In Table 1, the location and extraction yields of *S. connatum* are given.

**Table 1.** The location and extraction yields of *S. connatum*
***Çizelge 1.*** *S. connatum'un ekstre verimleri ve lokasyon bilgileri*

| Plant material *Bitki materyali* | Location *Lokasyon* | Part *Kısım* | Extraction methods *Ekstraksiyon metodları* | Yield (%) *Verim(%)* |
|---|---|---|---|---|
| *S. connatum* | C4 Adana, Pozantı, Pozantı Ulukışla yolu 17. km, roadside slopes, 29.05.2017, S.Doğu 3290 & Y. Bağcı (C4 Adana, Pozantı, Pozantı Ulukışla yolu 17. km, yol kenarı yamaçlar, 29.05.2017, S.Doğu 3290 & Y. Bağcı) | Aerial parts Toprak üstü | Soxhlet extractor Soxhlet ekstraktörü | 24.16 |
| | | | Ultrasound assisted Ultrasonik destekli | 22.82 |
| | | | Maceration Maserasyon | 21.4 |
| | | Roots Kök | Soxhlet extractor Soxhlet ekstraktörü | 57.26 |
| | | | Ultrasound assisted Ultrasonik destekli | 31.73 |
| | | | Maceration Maserasyon | 8.07 |

## Antioxidant assay

### Determination of Total Phenolic Content (TPC)

The spectrophotometric Folin-Ciocalteu (F-C) technique was used to evaluate the TPC in the extracts of *S. connatum* aerial parts and roots, with some modifications to Clarke's et al methods (Clarke et al., 2013). 10 µL of extract, suitably diluted with DMSO, were combined with 100 µL of freshly 10-fold diluted F–C reagent in distilled water. After 5 minutes, 100 µL of $Na_2CO_3$ were added to the mixture.

### Determination of Total Flavonoid Content (TFC)

The total flavonoid concentration in *S. connatum* aerial parts and roots extracts was determined using the aluminum chloride colorimetric technique (Yang et al., 2012a). On a 96-well plate, the test solution (150 µL) produced with ethanol was mixed with 2% $AlCl_3$. In a microplate reader, the absorbance was measured at 435 nm. On a dry weight basis, the total flavonoid concentration was estimated as quercetin equivalent /g extract (mg QE/g).

### DPPH radical scavenging activity

Free radical scavenging ability of the extracts was tested by DPPH radical scavenging assay as described by Eruygur et al. (2019). In a 96-well plate, 180 µL of DPPH solution were mixed with MeOH after 20 µL of test solution. After 15 minutes of incubation in the dark, the plate was measured at 540 nm with an Elisa reader using (Multiscan Sky, USA) (Eruygur et al., 2019). As a positive control, ascorbic acid was employed. The results were expressed as mean standard deviation (SD). The findings were expressed as a percentage of DPPH scavenging efficiency:

*% DPPH Scavenging Effect = (Control Absorbance – Sample Absorbance)/Control Absorbance* × 100. (1)

### ABTS

According to Re et al., ABTS cation radical decolorization activity of the extracts was done (Re et al., 1999) with modest modifications. The stock solution of ABTS+ radical was prepared by allowing 15 mL of 7 mM ABTS and 264 µL of 140 mM potassium persulfate solution to stand in the dark at room temperature for 16 h before the assay. The ABTS working solution was freshly produced by diluting the stock solution with 80% MeOH and measuring the absorbance to give 0.70 ± 0.02 at 734 nm. Sample solution were combined with ABTS working solution in a 96-well plate. For comparison of the ABTS+ scavenging activity, ascorbic acid was utilized as an antioxidant standard. The following equation was used to calculate the percent ABTS scavenging effect:

*% ABTS Scavenging Effect = (Control Absorbance – Sample Absorbance)/Control Absorbance* × 100. (2)

### Iron-chelating activities

The iron chelating activity of the extracts was

assessed by the interaction of ferrozin-$Fe^{2+}$ complex, according to Chai et al. (Chai et al., 2014). In all, 0.4 mL of 0.2 mM ferrozine, 0.2 mL of 0.1 mM $FeSO_4$, and 0.2 mL of extract in different concentrations were mixed and incubated for 10 mins at room temperature. The absorbances were recored at 562 nm. EDTA was employed as a positive control.

### Enzyme inhibition assay

#### Acetylcholinesterase and butrylcholinesterase inhibition

This experiment was conducted according to the method fo Ellman et al. with some modifications (Šinko et al., 2007). At 25 °C, a mixture of 20 µL of test sample/reference standard, 140 µL of 200 mM phosphate buffer (PBS buffer) (pH 7.7), 10 µL of 5,5-ditio-bis-2-nitrobenzoic acid (DTNB), and 20 µL of enzyme (0.22 U $mL^{-1}$ for acetylcholinesterase 0.1 U $mL^{-1}$ for butyrylcholinesterase produced in PBS buffer). Following the addition of 10 µL of 0.5 mM DTNB, 10 µL of substrate (0.2 mM butyrylthiocholine iodide/ 0.71 mM acetylthiocholine iodide) was mixed and incubated for another 5 minutes. When the substrate was added, the absorbance was measured at 0 and 5 minutes at 412 nm. As a positive control, galantamine hydrobromide was used. The following equation was used to express the findings:

*% Inhibition= [(Absorbance of control$_{0\text{-}5}$min – Absorbance of test sample$_{0\text{-}5}$min)/Absorbance of control$_{0\text{-}5}$min]* ×100 (3)

#### Tyrosinase (TYR)inhibition

20 µL of sample solution was diluted with 80% methanol, 100 µL of phosphate buffer (0.1 M), and 20 µL of mushroom TYR (250 U $mL^{-1}$) were combined in a 96 well plate and incubated for about 10 minutes. After adding 20 µL of 3 mM L-tyrosine as a substrate,the mixture was further incubated for 30 minutes. The absorbance was measured at 492 nm (Yang et al., 2012b). Kojic acid was employed as positive control. The inhibitory effects of the extracts on mushroom TYR was calculated by the following formula:

*% Inhibition= [(Absorbance of control– Absorbance of test sample)/Absorbance of control]* ×100 (4)

## RESULTS

### Antioxidant activity

The total phenol content of the extracts was measured as mg gallic acid equivalent (GAE) $g^{-1}$ extract, while mg quercetin equivalent (QUE) $g^{-1}$ extract was used to calculate total flavonoid content. In Figure 1, total phenol and flavonoid contents of *S. connatum* extracts prepared by soxhlet extractor, ultrasonic assisted extraction, and maceration procedures are given. While the total phenol content of the methanol extract with maceration was found as the highest in the extracts of *S. connatum* roots, ultrasonic assisted method was determined as the highest one in the extracts of *S. connatum* aerial parts. The highest phenolic content was detected in the methanol extract of *S. connatum* aerial parts (133,77±6,69 mg GAE g-1) with ultrasonic assisted method. The methanol extract of the roots of *S.connatum* was found to have lower total flavonoid content than aerial parts using ultrasonic assisted methods. Among the aerial parts of the plant extracts, the highest flavonoid content (124.19±4.24 mg QUE g-1) was determined in the methanol extract prepared by ultrasonic assisted method. Total flavonoid content in the aerial parts of the plant extract prepared by maceration could not be measured because of the the nondetermination of the absorbance. In addition, it was measured that the extract of aerial parts has higher phenolic compounds than the extract of roots.

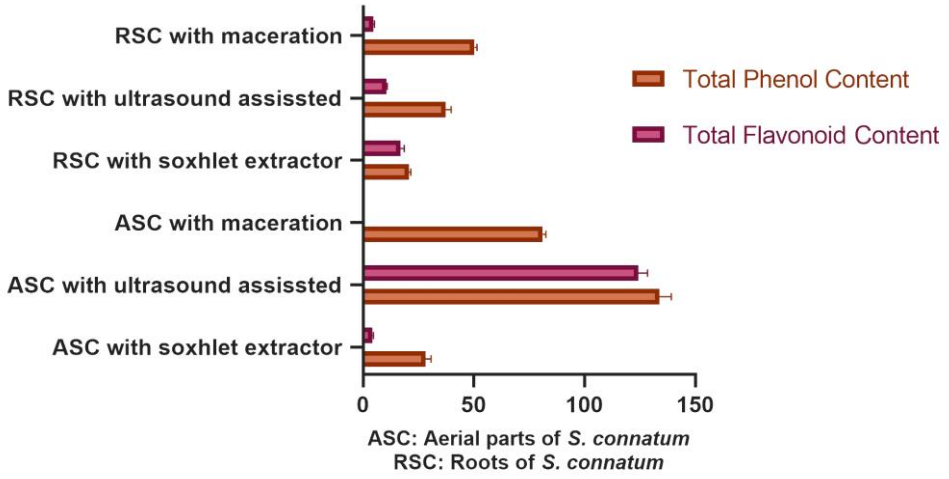


**Figure 1.** Total phenol and total flavonoid contents of the methanol extracts of *S. connatum*
***Şekil 1 .*** *S. connatum metanol ekstrelerinin total fenol ve flavonoit içerikleri*

The methanol extracts of *S. connatum* aerial parts and roots were examined for *in vitro* antioxidant activities such as DPPH and ABTS radical scavenging effects, as well as iron chelating capacities (Table 2). All antioxidant activity assays were proceeded in quadruplicate, and the findings were expressed as mean ± standard deviation (SD). Inhibition values of the extracts were given in Figure 2-4 at different concentrations. In antioxidant activity on DPPH, ASC with ultrasound assisted ($IC_{50}$= 32.28±1.22 µg $mL^{-1}$) and ASC with maceration ($IC_{50}$= 49.63±2.17 µg $mL^{-1}$) showed the high antioxidant activities. Aerial parts and root extracts of *S. connatum* prepared with soxhlet extractor had the low antioxidant activities with 15.41, 9.38 % inhibitor values, respectively. The methanol extracts prepared by maceration and ultrasonic assisted techniques from *S. connatum* roots ($IC_{50}$=157.65±2.47, 158.40±2.55 µg $mL^{-1}$, respectively), and aerial parts ($IC_{50}$= 100.17±5.77 µg $mL^{-1}$; $IC_{50}$= 19.18±1.69 µg $mL^{-1}$) have higher iron chelating ability than the the roots and aerial parts extracts with soxhlet extractor. When ABTS scavenging activity was evaluated, ASC and RSC with soxhlet extractor showed moderate activity, while other extracts exhibited similar activity using ascorbic acid as a positive control.

## Enzyme inhibition activity

In enzyme inhibition activity assays, the highest inhibitory activity against both AChE ($IC_{50}$= 57.47±2.81 µg $mL^{-1}$) and BuChE ($IC_{50}$= 38.91±5.31 µg $mL^{-1}$) was observed in the extract of ASC with ultrasound assissted. Similar activities against BuChE were observed in the aerial parts ($IC_{50}$= 58.53±4.63 µg $mL^{-1}$) and roots extracts ($IC_{50}$= 58.72±2.44 µg $mL^{-1}$) with soxhlet extractor.

Remarkable inhibitory activity was also determineted against AChE in extracts with soxhlet extractor. and $IC_{50}$ values were found in aerial parts($IC_{50}$= 65.83±5.66 µg $mL^{-1}$) and root extracts ($IC_{50}$= 86.86±13.79 µg $mL^{-1}$), respectively. RSC with maceration have moderate and lower activity than

**Table 2.** ABTS, and DPPH radical scavenging effects, and iron chelating activities of the extracts of *S. connatum*
***Çizelge 2.*** *S. connatum ekstrelerinin ABTS ve DPPH radikal süpürücü etkileri ve demir şelasyon aktiviteleri*

| **Methods** **Metodlar** | **ABTS (Inhibition percentage± S.D. %[a])** ***(İnhibisyon Yüzde ± S.D.[a])*** **$IC_{50}$: µg $mL^{-1}$** 166.666 µg $mL^{-1}$ [b] | **DPPH (Inhibition percentage± S.D. %[a])** ***(İnhibisyon Yüzde ± S.D.[a])*** **$IC_{50}$: µg $mL^{-1}$** 50 µg $mL^{-1}$ [b] | **Iron-chelating activities (Inhibition percentage± S.D. %[a])** ***(İnhibisyon Yüzde ± S.D.[a])*** **$IC_{50}$: µg $mL^{-1}$** 125 µg $mL^{-1}$ [b] |
|---|---|---|---|
| ASC with soxhlet extractor *Soxhlet ekstraktörü ile ASC* | 41.53±3.67 ($IC_{50}$: 187.2±0.85) | 15.41±0.55 | 8.28±2.60 |
| ASC with ultrasound assisted *Ultrasonik destekli ile ASC* | 85.85±0.20 ($IC_{50}$: 35.36±2.79) | 69.18±2.13 ($IC_{50}$: 32.28±1.22) | 78.56±0.73 ($IC_{50}$: 19.18±1.69) |
| ASC with maceration *Maserasyon ile ASC* | 87.37±1.62 ($IC_{50}$: 52.15±3.57) | 48.90±1.79 ($IC_{50}$: 49.63±2.17) | 59.38±4.91 ($IC_{50}$: 100.17±5.77) |
| RSC with soxhlet extractor *Soxhlet ekstraktörü ile RSC* | 52.21±4.38 ($IC_{50}$: 189.37±3.59) | 9.38±1.25 | 17.08±2.53 |
| RSC with ultrasound assisted *Ultrasonik destekli ile RSC* | 85.41±0.48 ($IC_{50}$: 96.5±5.39) | 22.18±1.54 ($IC_{50}$: 121.37±2.37) | 40.25±1.70 ($IC_{50}$: 158.40±2.55) |
| RSC with maceration *Maserasyon ile RSC* | 77.10±2.85 ($IC_{50}$: 104.3±3.70) | 19.48±0.61 ($IC_{50}$: 148.25±5.30) | 35.65±2.80 ($IC_{50}$: 157.65±2.47) |
| References *Referanslar* | 87.51±0.17[c] | 93.91±0.14[c] | 87.06±0.34[d] |

[a]Standard deviation [b]Final concentration [c]Ascorbic acid (2 mg $mL^{-1}$) [d]EDTA (2 mg $mL^{-1}$)

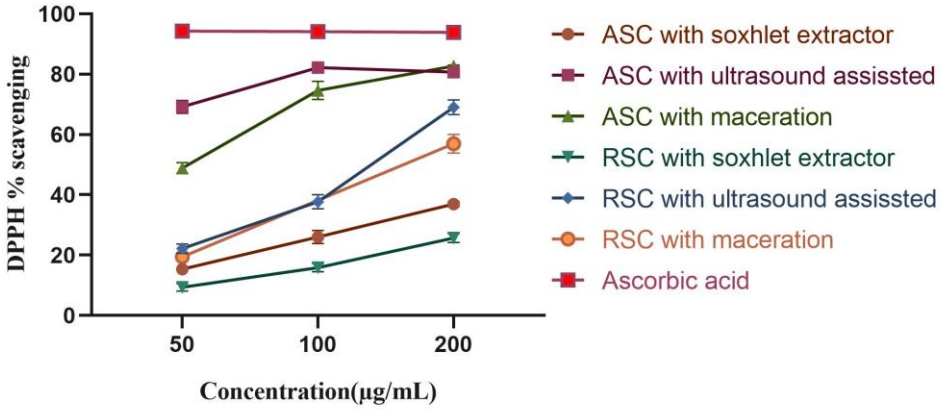


**Figure 2.** DPPH radical scavenging activity of the methanol extracts of *S. connatum*
***Şekil 2.*** *S. connatum metanol ekstrelerinin DPPH radikal süpürücü aktiviteleri*

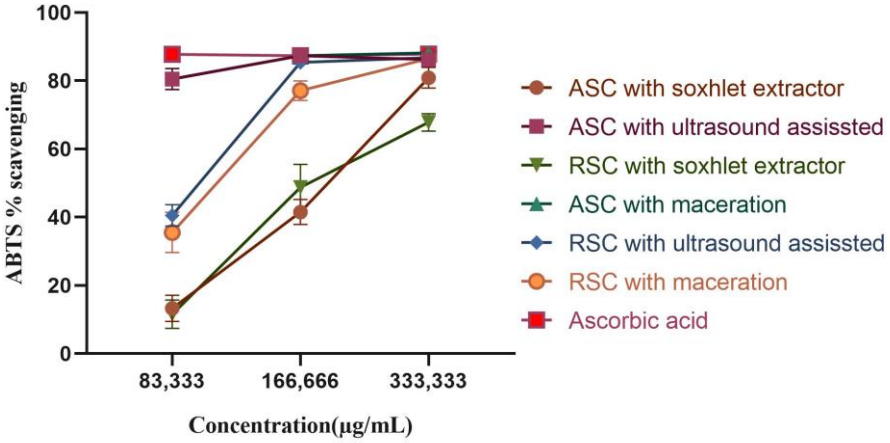


**Figure 3.** ABTS radical scavenging activity of the methanol extracts of *S. connatum*
***Şekil 3.*** *S. connatum metanol ekstrelerinin ABTS radikal süpürücü aktiviteleri*

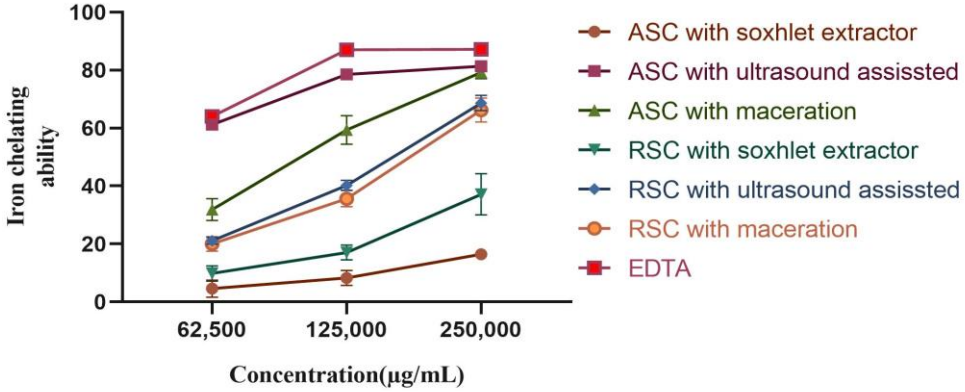


**Figure 4.** Iron-chelating activities of the methanol extracts of *S. connatum*
***Şekil 4.*** *S. connatum metanol ekstrelerinin demir şelasyon aktiviteleri*

other extracts against AChE ($IC_{50}$= 147.0±11.17 µg $mL^{-1}$) and BuChE ($IC_{50}$=144.68±5.19 µg $mL^{-1}$). All of the extracts showed moderate inhibitory activity against TYR. Similar activities were found in *S. connatum* root extracts prepared with three different methods. The RSC extract with ultrasound assisted had the smallest $IC_{50}$ value and was the most effective extract ($IC_{50}$= 189.90±3.82 µg $mL^{-1}$) than other extract.

The $IC_{50}$ values of the extracts are summarized in Table 3.

**Table 3.** Enzyme inhibitory activities of the methanol extracts of *S. connatum*
***Çizelge 3.*** *S. connatum metanol ekstrelerinin enzim inhibitör aktiviteleri*

| **Methods** **Metodlar** | **AChE (Inhibition percentage± S.D. %[a])** *(İnhibisyon Yüzde ± S.D.[a])* **$IC_{50}$: µg $mL^{-1}$** 200 µg $mL^{-1}$ [b] | **BuChE (Inhibition percentage± S.D. %[a])** *(İnhibisyon Yüzde ± S.D.[a])* **$IC_{50}$: µg $mL^{-1}$** 200 µg $mL^{-1}$ [b] | **TYR (Inhibition percentage± S.D. %[a])** *(İnhibisyon Yüzde ± S.D.[a])* **$IC_{50}$: µg $mL^{-1}$** 250 µg $mL^{-1}$ [b] |
|---|---|---|---|
| ASC with soxhlet extractor *Soxhlet ekstraktörü ile ASC* | 73.83±3.30 ($IC_{50}$: 65.83±5.66) | 75.54±2.59 ($IC_{50}$: 58.53±4.63) | 46.13±1.96 |
| ASC with ultrasound assisted *Ultrasonik destekli ile ASC* | 93.10±2.09 ($IC_{50}$: 57.47±2.81) | 83.81±7.63 ($IC_{50}$: 38.91±5.31) | 34.11±1.02 |
| ASC with maceration *Maserasyon ile ASC* | 74.68±3.83 ($IC_{50}$: 117.3±0.78) | 69.96±4.60 ($IC_{50}$: 74.83±4.28) | 44.31±2.32 |
| RSC with soxhlet extractor *Soxhlet ekstraktörü ile RSC* | 75.09±8.42 ($IC_{50}$: 86.86±13.79) | 99.82±6.23 ($IC_{50}$: 58.72±2.44) | 54.53±7.58 ($IC_{50}$: 213.40±7.92) |
| RSC with ultrasound assisted *Ultrasonik destekli ile RSC* | 83.64±0.90 ($IC_{50}$: 75.90±2.70) | 61.87±2.03 ($IC_{50}$: 119.10±3.82) | 54.49±5.65 ($IC_{50}$: 189.90±3.82) |
| RSC with maceration *Maserasyon ile RSC* | 56.45±4.10 ($IC_{50}$: 147.0±11.17) | 62.83±2.91 ($IC_{50}$: 144.68±5.19) | 54.87±3.78 ($IC_{50}$: 198.40±3.25) |
| References *Referanslar* | 99.10±1.18[c] | 84.34±4.85[c] | 80.96±0.51[d] |

[a]Standard deviation [b] Final concentration [c] Galanthamine hydrobromür [d] Kojic acid

## DISCUSSION

Extraction is a crucial stage in the process of discovering bioactive components in medicinal plants. The biological activity of plant extracts differed significantly depending on the extraction method used, highlighting the necessity of choosing the right extraction process (Murugan and Parimelazhagan, 2014). In this study, significant differences were found in both enzyme inhibition and antioxidant determinations between plant extracts prepared using different extraction methods. By evaluating the yield differences in the extraction methods used, it is predicted that the content of the extracts may be different. The yield of the prepared root extraction in soxhlet extraction (57%) is considerably higher than the others.

In a study by Minareci et al., the antioxidant activity of the *S. connatum* aerial parts extract prepared by maceration was evaluated by the DPPH radical scavenging method. DPPH radical scavenging activity was observed as 92.51 ± 0.09% at 1.09 mg $mL^{-1}$concentration (Minareci and Kalyoncu, 2012). ABTS radical scavenging activity, iron chelation activity, total phenol, and total flavonoid contents studies have not been performed on *S. connatum* before, and they were conducted for the first time. In this study, the biological activities of the extracts obtained from different parts of the *S. connatum* prepared using three different methods were evaluated. Total phenol contents of roots and aerial parts with soxhlet were lower than extracts prepared with other methods. *S. connatum* aerial parts has a much higher total phenol and total flavonoid content than its roots. For this reason, *S. connatum* aerial parts generally may have been found to be more active in antioxidant activity assay.

Enzyme inhibition studies have not been performed on *S. connatum* and this study was carried out for the first time. In studies on different *Smyrnium* species; it was determined that *S. olansatrum* has low inhibitory activity against AChE, BuChE, and TYR (Orhan et al., 2016). In another study, it was determinated that the methanol extract of *S. cordiifolium* have high cholinesterase inhibitory actitviy (2.92 and 2.64 mg galantamine equivalent/g extract, for acetyl- and butyrylcholinesterase, respectively) and good TYR inhibitory activity (137.54 mg kojic acid equivalent/g extract) (Zengin et

al., 2019). In this study, it was observed that extracts of *S. connatum* aerial parts and roots showed high inhibition activity against both cholinesterase's and moderate inhibition activity against TYR. Also, it was determined that ASC with ultrasound assisted extract had the highest antioxidant and cholinesterase inhibitory activity.

## CONCLUSION

The biological activity of the aerial parts of *S. connatum* generally was found to be more active than its roots. We discovered that the enzyme inhibitors and antioxidant capacity we investigated were effective in treating neurodegenerative disorders. As a result of the enzyme inhibition and antioxidant activity studies, the *S.connatum* deserves to performing further *in vivo* biological activity and phytochemical analysis studies.

### Author Contributions

The plant material belonging to this study was collected by Y.B. and S.D., and the species identification was carried out by Y.B. In order to carry out the study, the facilities of the Faculty of Science and the Faculty of Pharmacy were used. Designing the study and deciding on the appropriate experimental methods were carried out by F.A., N.E., and T.D. Experimental analyzes of the study were proceeded by F.A., N.E., and T.D. Article draft text was written by T.D. with the supervision of F.A. The manuscript was finalized with the critical feedback on the study, analyzes and article provided by F.A., Y.B., S.D., and N.E.

### Conflict of Interest

The authors declare that they do not have any competition and any conflicts of interest.

## REFERENCES

Abbasi N, Rafieian-Kopaei M, Karami N, Ghanadi K 2019. An Ethnobotanical Study of Hepatoprotective Herbs from Shahrekord, Chaharmahal and Bakhtiari Province, Southwest of Iran. EJVS 50 (2): 129-134.

Alam J, Sharma L 2019. Potential Enzymatic Targets in Alzheimer's: A Comprehensive Review. Curr Drug Targets 20(3): 316-339.

Amiri MS, Joharchi MR 2016. Ethnobotanical Knowledge of Apiaceae Family in Iran: A review. Avicenna J Phytomedicine 6(6): 621-635.

Anand P, Singh B 2013. A Review on Cholinesterase Inhibitors for Alzheimer's Disease. Arch Pharm Res 36(4): 375-399.

Bennour N, Mighri H, Eljani H, Zammouri T, Akrout A 2020. Effect of Solvent Evaporation Method on Phenolic Compounds and the Antioxidant Activity of *Moringa oleifera* Cultivated in Southern Tunisia. S Afr J Bot 129: 181-190.

Bermejo JEH, León J. 1994. Neglected crops: 1492 from a different perspective. Plant Production and Protection Series No. 26, FAO, Rome, Italy, 320 p.

Bulut G, Tuzlaci E 2013. An Ethnobotanical Study of Medicinal Plants in Turgutlu (Manisa-Turkey). J Ethnopharmacol 149(3): 633-647.

Caprioli G, Fiorini D, Maggi F, Marangoni M, Papa F, Vittori S, Sagratini G 2014. Ascorbic Acid Content, Fatty Acid Composition and Nutritional Value of the Neglected Vegetable Alexanders (*Smyrnium olusatrum* L., Apiaceae). J Food Compost Anal 35(1): 30-36.

Chai T, Mohan M, Ong H, Wong F 2014. Antioxidant, Iron-chelating and Anti-glucosidase Activities of *Typha domingensis* Pers (Typhaceae). Trop J Pharm Res 13(1): 67-72.

Clarke G, Ting KN, Wiart C, Fry J 2013. High Correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging, Ferric Reducing Activity Potential and Total Phenolics Content Indicates Redundancy in Use of All Three Assays to Screen for Antioxidant Activity of Extracts of Plants from the Malaysian Rainforest. Antioxidants 2(1): 1-10.

Davis PH 1972. Flora of Turkey and East Aegean Island. Edinburg University Press, Edinburgh, 338-339 p.

Demirci S, Özkan EE, Demirci S 2014. Ethnobotanical Studies of Some Apiaceae Plants in Kahramanmaras and A review of Their Phytochemical Studies. Istanbul J Pharm 44(2): 241-250.

Eruygur N, Ucar E, Akpulat HA, Shahsavari K, Safavi SM, Kahrizi D 2019. In vitro Antioxidant Assessment, Screening of Enzyme Inhibitory Activities of Methanol and Water Extracts and Gene Expression in *Hypericum lydium*. Mol Biol Rep 46(2): 2121-2129.

Minareci E, Kalyoncu F 2012. Proximate Composition, Antimicrobial and Antioxidant Activities of Six Wild Edible Celeries (*Smyrnium* L.). AJPP 6(13): 968-972.

Mungan F, Yıldız K, Kılıç M, Kuh M 2008. A Morphological Study of *Smyrnium* Apiaceae from Turkey. Biodivers Conserv 8(3): 54-59.

Mungan F, Yildiz K, Minareci E, Kiliccedil M 2011. A Palynological Study of the Genus *Smyrnium* (Umbelliferae) from Turkey. J Med Plant Res 5(6): 997-1003.

Murugan R, Parimelazhagan T 2014. Comparative Evaluation of Different Extraction Methods for Antioxidant and Anti-inflammatory Properties from *Osbeckia parvifolia* Arn.–An In vitro Approach. J King Saud Univ Sci 26(4): 267-275.

Orhan IE, Tosun F, Skalicka-Woźniak K 2016. Cholinesterase, Tyrosinase Inhibitory and

Antioxidant Potential of Randomly Selected Umbelliferous Plant Species and Chromatographic Profile of *Heracleum platytaenium* Boiss. and *Angelica sylvestris* L. var. *sylvestris*. J Serbian Chem Soc 81(4): 357-368.

Pillaiyar T, Manickam M, Namasivayam V 2017. Skin Whitening Agents: Medicinal Chemistry Perspective of Tyrosinase Inhibitors. J Enzyme Inhib Med Chem 32(1): 403-425.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C 1999. Antioxidant Activity Applying An Improved ABTS Radical Cation Decolorization Assay. Free Radic Biol Med 26(9-10): 1231-1237.

Šinko G, Čalić M, Bosak A, Kovarik Z 2007. Limitation of the Ellman Method: Cholinesterase Activity Measurement in the Presence of Oximes. Anal Biochem 370(2): 223-227.

Stevens PF 1972. *Smyrnium* L. (Flora of Turkey and East Aegean Island, Edinburg University Press, Edinburg: Ed. Davis PH), vol. 4, 337-341.

Yang Z, Wang Y, Wang Y, Zhang Y 2012a. Bioassay-guided Screening and Isolation of α-Glucosidase and Tyrosinase Inhibitors from Leaves of *Morus alba*. Food Chem 131(2): 617-625.

Yang Z, Zhang Y, Sun L, Wang Y, Gao X, Cheng Y 2012b. An Ultrafiltration High-performance Liquid Chromatography Coupled with Diode Array Detector and Mass Spectrometry Approach for Screening and Characterising Tyrosinase Inhibitors from Mulberry Leaves. Anal Chim Acta 719: 87-95.

Zengin G, Mahomoodally MF, Paksoy MY, Picot-Allain C, Glamocilja J, Sokovic M, Diuzheva A, Jekő J, Cziáky Z, Rodrigues MJ 2019. Phytochemical Characterization and Bioactivities of Five Apiaceae Species: Natural Sources for Novel Ingredients. Ind Crops Prod 135: 107-121.

Zhang YJ, Gan RY, Li S, Zhou Y, Li AN, Xu DP, Li HB 2015. Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. Molecules 20(12): 21138-21156.