Evaluation of the Antioxidant Potency of Seseli L. Species (Apiaceae)

Seseli L. Türlerinin (Apiaceae) Antioksidan Potansiyellerinin Değerlendirilmesi

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ABSTRACT

Objectives: In the present study, the antioxidant potency of ethyl acetate (AcOEt) and methanol (MeOH) extracts from the aerial parts of Seseli L. species was investigated for the first time.

Materials and Methods: Seseli species L. such as Seseli andronakii Woronow ex Schischk., S. campestre Besser, S. corymbosum Boiss. & Heldr., S. gummiferum subsp. gummiferum Pall. ex Sm., S. hartvigii Parolly & Nordt, S. ibanotis (L.) W.Koch, S. petraeum M.Bieb., S. peucedanoides (M.Bieb.) Koso-Pol., S. resinosum Freyn & Sint., and S. tortuosum L. growing in Turkey were collected and evaluated for their antioxidant capacity by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and lipid peroxidation (LPO) inhibition methods.

Results: The highest activities as a scavenger of DPPH radicals were found in the AcOEt extracts of S. peucedanoides (M.Bieb.) Koso-Pol (IC50=0.49 mg/mL), and S. libanotis (IC50=0.75 mg/mL); α-tocopherol was used as a positive control. On the other hand, in the LPO assay, the highest activities were determined in AcOEt and MeOH extracts (at 5 mg/mL) of S. tortuosum and S. libanotis (84-94%).

Conclusion: This report gives important information about the antioxidant capacity of Seseli L. species. This research on antioxidant capacity proves that the use of some species used in Eastern Anatolia (in salads) is correct. With this screening study performed in Seseli L. species growing in Turkey, in the future, it is planned to isolate antioxidant compounds from the most active strains of Seseli L.

Key words: Antioxidant, Apiaceae, DPPH, LPO, Seseli

ÖZ

Amaç: Bu çalışmada, ilk kez Seseli L. türlerinin toprak üstü kısımlarından elde edilen, etil asetat (AcOEt) ve metanol (MeOH) ekstrelerinin antioksidan potansiyelleri araştırılmıştır.

Gereç ve Yöntemler: Türkiye’de yetişen bazı Seseli L. türlerinin, Seseli andronakii Woronow ex Schischk., S. campestre Besser, S. corymbosum Boiss. & Heldr., S. gummiferum subsp. gummiferum Pall. ex Sm., S. hartvigii Parolly & Nordt, S. ibanotis (L.) W.Koch, S. petraeum M.Bieb., S. peucedanoides (M.Bieb.) Koso-Pol., S. resinosum Freyn & Sint., ve S. tortuosum L. yetiştirildiği yörelerden toprak üstü kısımlarından elde edilen, etil asetat (AcOEt) ve metanol (MeOH) ekstrelerinin antioksidan potansiyelleri değerlendirilmiştir.

Bulgular: En yüksek radikal süpürücü etkinin Seseli peucedanoides (M.Bieb.) Koso-Pol (IC50=0.49 mg/mL) ve Seseli libanotis (IC50=0.75 mg/mL) etil asetat ekstrelerinde olduğu tespit edilmiştir. Diğer yandan, lipid peroksidasyonu (LPO) inhibisyon yöntemlerinde ise S. tortuosum (84-94%) ve S. libanotis (94-95%) etil asetat ve metanol (5 mg/mL) ekstrelerinde en yüksek inhibisyon değerleri tespit edilmiştir.

Sonuç: Bu çalışma, Seki L. türlerinin antioksidan kapasitesi hakkında önemli bilgiler vermiştir. Bu araştırmada, etil asetat ve metanol ekstrelerinin antioksidan potansiyelleri değerlendirilmiş ve en yüksek antioksidan etkileri Seseli peucedanoides (M.Bieb.) Koso-Pol ve Seseli libanotis (S. I. L.) w. koç, S. petraeum M.Bieb., S. peucedanoides (M.Bieb.) Koso-Pol., S. resinosum Freyn & Sint., and S. tortuosum L. türlerinde elde edilmiştir. Bu çalışmanın sonucunda, S. tortuosum ve S. libanotis türlerinde elde edilen inhibisyon oranları, AcOEt ve MeOH ekstrelerinde 84-94% ve 84-95% olarak elde edilmiştir.

Anahtar kelimeler: Antioksidan, Apiaceae, DPPH, LPO, Seseli

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INTRODUCTION

The Apiaceae (previously Umbelliferae) is a well-known family in the plant kingdom with aromatic plants and economically important species. Some members of the family are used as foods, spices, condiments, and ornaments. The genus Seseli L. belongs to the family Apiaceae and is distributed in Asia and Europe, comprising more than 12 taxa in Turkey, of which 4 are native to the region. In addition, new species have recently been discovered. Moreover, the latest taxonomy of the type section of the genus Seseli has been given based on the molecular data with recently updated names. Seseli is an ancient Greek name given to some individual members of the family Apiaceae by Hippocrates. Seseli species are mainly rich in coumarins as well as terpenoids, essential oils, and have many important pharmacological activities with healing effects such as in inflammation, swelling, rheumatism, pain, and the common cold. On the other hand, the fruit of S. indicum has been reported to have anthelmintic, carminative, stomachic, and stimulant properties. S. sibiricum is used for blending beverages and as a medicine for livestock in Kashmir. In addition, the fruit of S. libanotis is a local remedy for blood pressure control in Pakistan, and its essential oil from the fruit has potent antimicrobial activity. While S. indicum exhibited strong insect repellent activity and fungitoxicity, the fruit of S. tortuosum is recorded to have emmenagogic and antiflatulent effects. Moreover, the leaves of S. libanotis (Kelemkeşir or Kelemenkeşir in Turkish) are consumed as a vegetable in salads and Eastern Turkey.

In Turkey, there are limited studies on Seseli species based on coumarins and essential oils. Previously, antimicrobial, anti-inflammatory, and antinociceptive effects have been examined in Turkish Seseli species. The plant kingdom presents secondary plant metabolites (especially polyphenols) as a wide range of natural antioxidants. The natural antioxidants in plants are of great interest in natural product science and many herbs have significant antioxidant potency. Antioxidants decrease oxidative stress in cells and are therefore very useful in the treatment of major degenerative diseases. The physiological role of antioxidant agents is to scavenge for free radicals in the case of overproduction of these reactive species.

Therefore, in the present study, we aimed to investigate the antioxidant potential of the aerial parts of Turkish Seseli species. The species were screened using in vitro 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and lipid peroxidation (LPO) inhibition assays.

MATERIALS AND METHODS

Plant material

Plant materials were collected from different localities in Turkey. All of the Seseli L. species were identified by Prof. H. Duman from the Department of Biology, Faculty of Science and Arts, and voucher specimens were deposited at the Herbarium of the Faculty of Pharmacy of Ankara University and the Herbarium of Gazi University, Ankara, Turkey. The species are listed in Table 1 (ethical committee approval and patient consent were not required).

Extraction of the plants

The extraction method in Fenglin et al. was used with some modifications. The aerial parts of each plant material, which were dried and powdered, were prepared according to the procedures described below:

- The ethyl acetate (AcOEt) extract: The plant material (10 g) was extracted with AcOEt at room temperature by a magnetic stirrer (x200 mL) for 24 hour. The extract was evaporated to dryness in a vacuum to give a crude AcOEt extract.

- The methanol (MeOH) extract: After the AcOEt extraction, the plant material (10 g) was extracted with MeOH (80%) at room temperature by a magnetic stirrer (x200 mL) for 24 hour. The extract was evaporated to dryness in vacuo to give a crude methanolic extract. The yields of all extracts are given in Table 2.

Table 1. Plant names and collection sites of Turkish Seseli L. species

| Species                        | Location                               | Herbarium no |
|-------------------------------|----------------------------------------|--------------|
| S. andronakii Woronow ex Schischk | Erzurum, Oltu-Sarikayalar, 1450-1750 m | ED 1617      |
| S. campestrum Besser          | İstanbul, Sultanbeyli, Paşaköy c. 500 m | ED 1656      |
| S. corymbosum Boiss. and Heldr. | Antalya-Akseki, Pınarbaşı village 1650-1900 m | AEF 21701   |
| S. gummiferum subsp. gummiferum Pall. ex Sm. | Ankara-Hassanoğlan, Idris mountain 1600-1700 m | AEF 21999   |
| S. hartvigii Parolly and Nordt | Antalya-Saklikent, Bakılar mountain, 2300-2500 m | AEF 21700   |
| S. libanotis (L.) W.Koch       | Ardahan-Posof, 1900 m                  | ED 1622      |
| S. petraeum M.Bieb.           | Gümüşhane, The road to Alemdar village, 1400 m | ED 1644      |
| S. peucedanoides (M.Bieb.) Koso-Polo | Ankara-Hassanoğlan, Idris mountain, 1600-1700 m | AEF 23158   |
| S. resinosum Freyn and Sint.  | Bartın-Çakraz, 0-5 m                   | AEF 21696    |
| S. tortuosum L.               | Ankara, Beynam forest, 1400 m          | ED 1612      |

AEF: Herbarium of the Faculty of Pharmacy of Ankara University
Ascorbic acid, thiobarbituric acid (TBA), DPPH, and α-tocopherol were purchased from Sigma Chemical Co (St. Louis, MO, USA).

Antioxidant capacity of the extracts

Radical scavenging capacity (DPPH)

The model of scavenging stable DPPH radicals is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. The reaction mixture contained 100 µM DPPH in MeOH and different concentrations of the crude extract. Absorbance at 517 nm was measured on a Shimadzu UV-1601 UV-VIS spectrometer at various concentrations (30 min after starting the reaction) at room temperature and the scavenging activity was calculated as the percentage of radical reduction. In our study, samples were dissolved in MeOH (80%) and AcOEt to 10 mg/mL and diluted to various concentrations. The scavenging activity was calculated as the percentage of radical reduction. The values of IC₅₀ were determined from a calibration curve for each plant extract. Each experiment was performed in triplicate. IC₅₀ values were determined from a calibration curve for each plant extract and α-tocopherol was used as the reference compound.

Assay of lipid peroxidation (LPO)

LPO was determined by a modified version of the method described by Mihara et al. It was measured spectrophotometrically by estimation of the TBA reactant substances (TBARS). Amounts of TBARS were expressed in nmol malondialdehyde/g tissue. A typical optimized assay mixture containing 0.5 mL of liver homogenate, 0.1 mL of Tris-HCl buffer (pH 7.2), 0.05 mL of 0.1 mM ascorbic acid, and 0.05 mL of 4 mM FeCl₂ and 0.05 mL of homogenate, 0.1 mL of Tris-HCl buffer (pH 7.2), 0.05 mL of 0.1 A typical optimized assay mixture containing 0.5 mL of liver homogenate, 0.1 mL of Tris-HCl buffer (pH 7.2), 0.05 mL of 0.1 mM ascorbic acid, and 0.05 mL of 4 mM FeCl₂ and 0.05 mL of homogenate, 0.1 mL of Tris-HCl buffer (pH 7.2), 0.05 mL of 0.1

RESULTS AND DISCUSSION

The present study deals with the radical scavenging activity (Table 3) and LPO (Table 4) of the AcOEt and MeOH extracts obtained from Seseli species growing in Turkey such as Seseli andronakii, S. campestre, S. corymbosum, S. gummiferum subsp. gummiferum, S. hartvigii, S. libanotis, S. petraeum, S. peucedanoides (M.Bieb.) Koso-Pol, S. resinosum, and S. tortuosum. The antioxidant activities of AcOEt and MeOH extracts obtained from the Seseli species were investigated by the DPPH scavenging and nonenzymatic rat hepatic microsomal LPO methods. In addition, their antioxidant activities were compared with those of the standard antioxidant α-tocopherol. The DPPH free radical scavenger assay is a simple and basic screening method for the discovery of bioactive substances. Free radicals are species that damage all the components of the body (lipids, proteins, DNA, etc.) and take part in mutations. In this case, antioxidants are important for body protection, helping reduce oxidative damage in the human body, and prevent LPO in foods.

| Species | AcOEt extract (w/w % mg) | MeOH extract (w/w % mg) |
|---------|--------------------------|-------------------------|
| SA      | 370                      | 154                     |
| SA      | 390                      | 154                     |
| SCa     | 1030                     | 108                     |
| SGG     | 870                      | 128                     |
| SH      | 330                      | 119                     |
| SL      | 270                      | 200                     |
| SP      | 750                      | 118                     |
| SPeu    | 310                      | 100                     |
| SR      | 420                      | 120                     |
| ST      | 570                      | 163                     |

**Table 2. The yield of extracts from Turkish Seseli L. species**

| Control | AcOEt extracts IC₅₀ (mg/mL) | MeOH extracts IC₅₀ (mg/mL) |
|---------|-----------------------------|---------------------------|
| SA      | 1.91±0.04                   | 0.125±0.003               |
| SH      | 1.94±0.03                   | 0.225±0.002               |
| ST      | 1.65±0.02                   | 0.205±0.005               |
| SL      | 0.75±0.07                   | 0.187±0.002               |
| SGG     | 3.07±0.04                   | 0.088±0.001               |
| SPeu    | 0.49±0.1                    | 0.091±0.004               |
| SR      | 1.18±0.15                   | 0.086±0.001               |
| SC      | 2.47±0.06                   | 0.253±0.005               |
| SCA     | 4.27±0.14                   | 0.185±0.008               |
| SP      | 0.172±0.006                 |                           |
| α-Tocopherol | 0.013±0.001               |                           |

SA: S. andronakii, SH: S. hartvigii, ST: S. tortuosum, SL: S. libanotis, SGG: S. gummiferum subsp. gummiferum, SPeu: S. peucedanoides, SR: S. resinosum, SC: S. corymbosum, SCa: S. campestre, SP: S. petraeum, AcOEt: Ethyl acetate, MeOH: Methanol
In our experiments, the results indicated that the extracts of some Turkish Seseli species have considerable effects on scavenging DPPH radicals (Figure 1). The AcOEt extract of S. peucedanoides (IC$_{50}$=0.49 mg/mL) and S. libanotis (IC$_{50}$=0.75 mg/mL) showed the most potent radical scavenging capacity (Table 3). These extracts were followed by S. resinosum (IC$_{50}$=1.18 mg/mL), S. tortuosum (IC$_{50}$=1.65 mg/mL), S. andronakii (IC$_{50}$=1.91 mg/mL), S. hartvigii (IC$_{50}$=1.94 mg/mL), S. corymbosum (IC$_{50}$=2.47 mg/mL), S. gummiferum subsp. gummiferum (IC$_{50}$=3.07 mg/mL), and S. campestre (IC$_{50}$=4.27 mg/mL) extracts.

The MeOH extracts of Seseli species have a higher DPPH radical scavenging effect than AcOEt extracts. The results showed that MeOH extracts of S. resinosum, S. gummiferum subsp. gummiferum, and S. peucedanoides have the highest scavenging capacity (IC$_{50}$=0.086, IC$_{50}$=0.088, and IC$_{50}$=0.091, respectively).

The TBA test results showed that MeOH extracts of Seseli spp. exhibited potent antioxidant effects (81-96% inhibition at 5 and 10 mg/mL concentrations) when compared to α-tocopherol. The AcOEt and MeOH extracts of S. tortuosum have the strongest anti-LPO activity (84-96% inhibition at a dose of 10 mg). The AcOEt and MeOH extracts of S. campestre, S. andronakii, and S. gummiferum subsp. gummiferum also exhibited a high anti-LPO effect in the LPO assay (Table 4).

### Table 4. Antilipid peroxidation effects of Seseli extracts

| Concentrations mg/mL | AcOEt extracts | MeOH extracts |
|----------------------|----------------|---------------|
| Control | AcOEt extracts | MeOH extracts | |
| b | AcOEt extracts | MeOH extracts | |
| SA | 2.5 | 0.148 | 34 | 5 | 0.027 | 88 |
| | 5 | 0.045 | 80 | 10 | 0.024 | 89 |
| SH | 2.5 | 0.084 | 63 | 5 | 0.026 | 88 |
| | 5 | 0.052 | 77 | 10 | 0.025 | 89 |
| ST | 2.5 | 0.102 | 55 | 5 | 0.011 | 95 |
| | 5 | 0.036 | 84 | 10 | 0.009 | 96 |
| SL | 2.5 | 0.222 | 1.2 | 5 | 0.037 | 83 |
| | 5 | 0.085 | 45 | 10 | 0.014 | 94 |
| SGG | 2.5 | 0.085 | 62 | 5 | 0.042 | 81 |
| | 5 | 0.039 | 82 | 10 | 0.035 | 84 |
| SPEu | 2.5 | 0.195 | 13 | 5 | 0.021 | 91 |
| | 5 | 0.129 | 43 | 10 | 0.022 | 90 |
| SR | 2.5 | 0.144 | 36 | 5 | 0.043 | 81 |
| | 5 | 0.049 | 78 | 10 | 0.026 | 88 |
| SC | 2.5 | 0.151 | 33 | 5 | 0.025 | 89 |
| | 5 | 0.067 | 70 | 10 | 0.018 | 92 |
| SCA | 2.5 | 0.088 | 61 | 5 | 0.025 | 89 |
| | 5 | 0.043 | 81 | 10 | 0.02 | 91 |
| SP | 2.5 | 0.156 | 31 | 5 | 0.028 | 87 |
| | 5 | 0.058 | 74 | 10 | 0.026 | 81 |
| α-Tocopherol | 0.22 | 0.009 | 96 | 2.5 | 0.009 | 96 |
| | 0.44 | 0.003 | 99 | 2.5 | 0.003 | 99 |

*Each value represents the mean ± standard deviation of 2-4 independent experiments, *AcOEt or MeOH only, control for extracts, *NE: No effect

SA: S. andronakii, SH: S. hartvigii, ST: S. tortuosum, SL: S. libanotis, SGG: S. gummiferum subsp. gummiferum, SPEu: S. peucedanoides, SR: S. resinosum, SC: S. corymbosum, SCA: S. campestre, SP: S. petraeum

Figure 1. Ethyl acetate extracts of Seseli species (1-10) and (11) α-tocopherol at various concentrations

(1) S. andronakii, (2) S. hartvigii, (3) S. tortuosum, (4) S. libanotis, (5) S. gummiferum subsp. gummiferum, (6) S. peucedanoides, (7) S. resinosum, (8) S. corymbosum, (9) S. campestre, (10) S. petraeum
In previous studies, the antioxidant potency of MeOH extract of *S. pallasii*, *S. libanotis* subsp. *libanotis*, and *S. libanotis* subsp. *intermedium* (aerial parts and fruits) was determined. *S. libanotis* subsp. *libanotis* showed the strongest antioxidant activity in the DPPH assay.\(^\text{54}\) Various extracts in different polarities from the roots, leaves, flowers, and fruit of *S. rigidum* were also studied, and the hexane extract of the root had the best effect among the other plant parts in the DPPH assay.\(^\text{55,56}\) In another study, the antioxidant activity of *Seseli rigidum* was evaluated in five extracts in different polarities (water, MeOH, acetone, ethyl acetate, and petroleum ether). The antioxidant effect of the aerial parts of the species was determined *in vitro* using DPPH reagent, and the highest antioxidant activity was expressed in water extract (46.15 µg/mL).\(^\text{57}\) Moreover, some of the compounds isolated from the methanolic extracts (80%) of *Seseli diffusum* have been found to have a strong antioxidant effect.\(^\text{58}\)

It is known that *Seseli* species contain phenolic compounds consisting mainly of coumarins,\(^\text{16}\) which have notable antioxidant potency.\(^\text{59-61}\) In addition, mostly oxygenated coumarins are accumulated in the AcOEt fractions, and the glycosides are present in the MeOH extract. The MeOH extract exhibits higher antioxidant activity, which may be explained by the presence of coumarin glucosides as highly polar compounds in the extract. The results show that there seems to be a good match between the content of the extracts and the antioxidant capacity. Finally, the activity might be due to the polar coumarins of the active *Seseli* species.\(^\text{30,62}\)

**CONCLUSION**

Natural products are generally known to be a good source of active compounds that have potential for the development of new therapeutic agents. The antioxidant properties of the AcOEt and MeOH extracts of *Seseli* species expressed as α-tocopherol equivalent antioxidant capacity were studied using DPPH and LPO assays. These results indicate that plant extracts prevent oxidative damage in normal cells due to their antioxidant properties. The best part of our research was that *Seseli* species growing in Turkey were screened for the first time for their antioxidant capacity. In addition, this research provides a scientific basis for the medicinal use of these plant materials. Therefore, we can conclude from the results of the present study that *Seseli* species may be a potential source of natural antioxidant compounds for the treatment of oxidative degeneration.

**Conflicts of interest: No conflict of interest was declared by the authors.**

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