EVALUATION of ANTIOXIDANT POTENCY on *SESELI* L. SPECIES (APIACEAE)

SESELI L. TÜRLERİNİN (APIACEAE) ANTİOKSİDAN POTANSİYELLERİNİN DEĞERLENDİRİLMESİ

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

METHODS: The *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* Parolty & Nordt, *S. libanotis* (L.) W. Koch, *S. petraeum* M. Bieb., *S. arenarium* M. Bieb., *S. resinosum* Freyn & Sint., *S. tortuosum* L. growing in Turkey were collected and evaluated for antioxidant capacity by using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method and LPO (lipid peroxidation inhibition) methods.

RESULTS: The highest activities as scavenger DPPH radical were found in the AcOEt extracts of *Seseli arenarium* (IC₅₀=0.49 mg/mL) and *S. libanotis* (IC₅₀=0.75 mg/mL), α -tocopherol used as 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%).

DISCUSSION AND CONCLUSION: This report is given considerable 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 is correct. With this screening study performed in *Seseli* L. species grown in Turkey, in the future, it is planned to isolate antioxidant compounds from the most active strains of *Seseli* L..

Keywords: Antioxidant, Apiaceae, DPPH, LPO, *Seseli*

INTRODUCTION

The Apiaceae (previously Umbelliferae) is a well-known family in the plant kingdom with aromatic plants and economically important species (Sayed-Ahmad et al., 2017). Some members of the family are used as foods, spices, condiments, and ornaments (Crowden et al., 1969; Lawrence, 1969; Pimenov and Leonov, 1993). The genus *Seseli* L. belongs to the Apiaceae family distributed in Asia, and Europe comprises more than 12 taxa in Turkey, of which 4 are native to the region (Hedge and Lamond, 1972; Davis et al., 1988; Duman, 2000; Parolty and Nordt, 2001). In addition, new species have recently been discovered (Pimenov and Kljuykov, 2000 and 2010; Güner and Duman, 2013; Çetin et al., 2015). Moreover, The latest

taxonomic type section of the *Seseli* genus have been performed based on the molecular data with recently updated names (Lyskov et al., 2018). *Seseli* is an ancient Greek name given to some individual members of the Apiaceae family by Hippocrates (Hamlyn, 1969). The *Seseli* species are mainly rich by coumarins besides terpenoids, essential oils, etc. (Barrero et al., 1990; Tosun and Özkal, 2003) and have many important pharmacological activities with healing effects such as in inflammation, swelling, rheumatism, pain and in the common cold (Hu et al., 1990). On the other hand, the fruits of *S. indicum* have been reported to have anthelmintic, carminative, stomachic and stimulant properties (Tandan et al., 1990). *S. sibiricum* is used for blending beverage and as a medicine for livestock in Kashmir (Austin et al., 1968). In addition, the fruits of *S. libanotis* were a local remedy for blood pressure control in Pakistan besides its essential oil from the fruits has potent antimicrobial activity (Syed et al., 1989). While *S. indicum* exhibited strong insect repellent activity (Dixit et al., 1992) and fungitoxicity (Chaturvedi & Tripathi, 1989); the fruits of *S. tortuosum* are recorded as emmenagogue and anti-flatulence (Baytop, 1999). Moreover, the leaves of *S. libanotis* (Kelemkeşir or Kelemenkeşir in Turkish) are consumed as a vegetable in salads in the Eastern of Turkey (Baytop, 1994).

In Turkey, there are limited studies on *Seseli* species based on the coumarins (Tosun et al., 2005; Tosun, 2006; Tosun et al., 2006a, Zhang et al., 2010; Shehzad et al., 2013) and the essential oils (Baser et al., 2000; Kaya et al., 2003; Tosun et al., 2005; 2006b; 2006c). Previously, antimicrobial (Tosun et al., 2004), anti-inflammatory and antinociceptive (Küpeli et al., 2005; Tosun et al., 2009; Chun et al., 2016) effects have been performed on Turkish *Seseli* species.

The plant kingdom presents secondary plant metabolites (especially polyphenols) as a wide range of natural antioxidants (Lee et al., 2003; Kim et al., 2010; Shahidi et al., 2018; Olas et al., 2018). The natural antioxidants in plants have a great interest in natural product science, and many herbs have significant antioxidant potency (Ng et al., 2000). Antioxidants decrease oxidative stress in cells and are therefore very useful in the treatment of major degenerative diseases (Krishnaiah et al., 2011). The physiological role of antioxidant compounds is to scavenge for free radicals (Muraina et al., 2009; Halliwell and Gutteridge, 1989) in case of the overproduction of these reactive species (Wong et al., 2006).

Therefore, in this study, we aimed to investigate the antioxidant potential of the aerial parts of Turkish *Seseli* species. Hereby, the species are screened by using *in*

vitro DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging and LPO (lipid peroxidation inhibition) assays.

MATERIALS AND METHODS

Plant material

Plant materials were collected from different localities of Turkey. All of the *Seseli* L. species were identified by Prof. H. Duman from Department of Biology, Faculty of Science & Art, Gazi University/Ankara/Turkey. Voucher specimens were deposited at the Herbarium of Faculty of Pharmacy of Ankara University (AEF) and the Herbarium of Gazi University (GAZI), Ankara, Turkey. The species are listed in Table 1.

Table1

Extraction of the plants

The extraction method in Fenglin et al. (2004) and Bthori et al. (2004) was used by some modifications. The aerial parts of each plant material, which are dried and powdered, were prepared following procedures as 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 h. The extract was evaporated to dryness in 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 h. The extract was evaporated to dryness in *vacuo* to give a crude methanolic extract. The yields of all extracts were given in Table 2.

Table2

Chemicals

Ascorbic acid (AA), thiobarbituric acid (TBA), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and α -tocopherol were purchased from Sigma Chemical Co (St Louis, MO).

Antioxidant capacity of the extracts

Radical scavenging capacity (DPPH)

DPPH free radical scavenging activity

The model of scavenging the stable DPPH radical 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 (Blois, 1958). The reaction mixture is contained 100 μ M DPPH in methanol 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 calculated as the percentage of the 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 IC_{50} were determined from a calibration curve for each plant extract. Each experiment was performed in triplicate. IC_{50} values were determined from a calibration curve for each plant extracts, and α -tocopherol was used as reference compound.

Assay of lipid peroxidation (LPO)

Lipid peroxidation was determined by the modified method of Mihara et al. (1980). Lipid peroxidation was measured spectrophotometrically by estimation of thiobarbituric acid-reactant substances (TBARS). Amounts of TBARS were expressed regarding nmol malondialdehyde (MDA)/g tissue. A typical optimized assay mixture contained 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, 0.05 mL of 4 mM $FeCl_2$ and 0.05 mL of various concentration of crude extract, or α -tocopherol were incubated for 1 h at 37 °C. After incubation, 3.0 mL of H_3PO_4 and 1 mL of 0.6 % TBA were added and shaken vigorously. The mixture was boiled for 30 min. After cooling, *n*-butanol was added, and the mixture was shaken vigorously. Then, the *n*-butanol phase was separated by centrifugation at 3000 rpm for 10 min. The absorbance of the supernatant was read at 532 nm against a blank, which contained all reagents except liver homogenate.

RESULTS AND DISCUSSION

The present study deals with the radical scavenging activity (Table 3), and lipid peroxidation (Table 4) of the AcOEt and MeOH extracts obtained from the *Seseli* L. species growing in Turkey such as *Seseli andronakii*, *S. campestre*, *S. corymbosum*, *S. gummiferum* subsp. *gummiferum*, *S. hartvigii*, *S. libanotis*, *S. petraeum*, *S. arenarium*, *S. resinosum*, *S. tortuosum*. The antioxidant activities of AcOEt and MeOH extracts obtained from *Seseli* species were investigated by the 1,1'-diphenyl-2-picrylhydrazyl (DPPH) scavenging, and non-enzymatic rat hepatic microsomal lipid peroxidation methods (LPO). In addition, their antioxidant activities were compared with 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 and damage to all the components of the body (lipids, proteins, DNA, etc.) and take place in mutations. In this case, antioxidants are important for body protection, which can help reduce oxidative damage in the human body and prevent lipid peroxidation in the foods (Liu & Ng, 2000; Ellnain-Wojtaszek et al., 2003).

In our experiments, the results indicated that the extracts of some Turkish *Seseli* species have considerable effects on scavenging DPPH radical (Figure 1). The AcOEt extract of *S. arenarium* ($IC_{50}=0.49$ 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), *S. campestre* ($IC_{50}=4.27$ mg/mL) extracts, respectively.

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

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

In the previous studies, the antioxidant potency of methanol extract for *S. pallasii*, *S. libanotis* ssp. *libanotis*, *S. libanotis* ssp. *intermedium* (aerial parts and fruits) have been determined. *S. libanotis* ssp. *libanotis* showed the strongest antioxidant activity in DPPH assay (Matejić et al., 2012). Various extracts in different polarities from roots, leaves, flowers, and fruits of *S. rigidum* were also studied, and the hexane extract of root had been found to be the best effect among the other plant parts in DPPH assay (Stojkovic et al., 2009; Stankov-Jovanović et al., 2015). In the other study, the antioxidant activity of *Seseli rigidum* was also evaluated on five extracts in different polarity (water, methanol, 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 greatest antioxidant activity was expressed in water extract (46.15 µg/mL) (Jakovljević et al., 2015). Also, some of the compounds isolated from the methanolic extracts (80%) of *Seseli diffusum* have been found to have a strong antioxidant effect (Abbaskhan et al., 2012).

It is known that the *Seseli* species contain phenolic compounds consisting mainly of coumarins (Tosun and Özkal, 2003) which have notable antioxidant potency (Torres et al., 2014; Pereira et al., 2018; Zhu et al., 2018). In addition, mostly oxygenated coumarins accumulated 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 seemed to be a good match between the content of the extracts and the antioxidant capacity. Eventually, the activity might be due to the polar coumarins of the active *Seseli* species (O'Kennedy & Thornes, 1997; Liu & Ng, 2000).

Figure 1.

Table 3.

Table 4.

CONCLUSION

Natural products are generally known to be a good source of active compounds which have the potential to develop new therapeutic agents. The antioxidant properties of the AcOEt and MeOH extracts of the *Seseli* species expressed as α -

tocopherol equivalent antioxidant capacity were studied using DPPH (1,1-diphenyl-2-picrylhydrazyl) and LPO (Lipid peroxidation) assays. These results indicate that plant extracts prevent oxidative damage in normal cells due to their antioxidant properties. The best part of our research, *Seseli* species growing in Turkey, have been 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 estimate from the results of the present study that the *Seseli* species may promise a potential source of natural antioxidant compounds for the treatment of oxidative degeneration.

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Table 1. Plant names and collection sites of Turkish <i>Seseli</i> L. species		
Species	Location	Herbarium no
<i>S. andronakii</i> Woronow ex Schischk	Erzurum, Oltu-Sarıkayalar, 1450-1750 m	ED 1617
<i>S. campestre</i> Besser	Istanbul, Sultanbeyli, Paşaköy c. 500m	ED 1656
<i>S. corymbosum</i> Boiss. & Heldr.	Antalya-Akseki, Pınarbaşı Village 1650-1900 m	AEF 21701
<i>S. gummiiferum</i> subsp. <i>gummiiferum</i> Pall. ex Sm.	Ankara-Hasanoğlu, İdris Mountain 1600-1700 m	AEF 21999
<i>S. hartvigii</i> Parolly & Nordt	Antalya-Saklıkent, Bakırlar Mountain, 2300-2500 m	AEF 21700
<i>S. libanotis</i> (L.) W. Koch	Ardahan-Posof, 1900 m	ED 1622
<i>S. petraeum</i> M. Bieb.	Gümüşhane, The road of Alemdar Village, 1400 m	ED 1644
<i>S. arenarium</i> M. Bieb.	Ankara-Hasanoğlu, İdris Mountain, 1600-1700 m	AEF 23158
<i>S. resinosum</i> Freyn & Sint.	Bartın-Cakraz, 0-5 m	AEF 21696
<i>S. tortuosum</i> L.	Ankara, Beynam Forest 1400 m.	ED 1612

Table 2. The yield of extracts from Turkish <i>Seseli</i> L. species		
Species	EtOAc extract (w/w % mg)	MeOH extract (w/w % mg)
<i>S. andronakii</i>	370	154
<i>S. campestre</i>	390	154
<i>S. corymbosum</i>	1030	108
<i>S. gummiferum</i> subsp. <i>gummiferum</i>	870	128
<i>S. hartvigii</i>	330	119
<i>S. libanotis</i>	270	200
<i>S. petraeum</i>	750	118
<i>S. arenarium</i>	310	100
<i>S. resinosum</i>	420	120
<i>S. tortuosum</i>	570	163

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

(1) SA: *S. andronakii*, (2) SH: *S. hartvigii*, (3) ST: *S. tortuosum*, (4) SL: *S. libanotis*,
 (5) SGG: *S. gummiferum* subsp. *gummiferum*, (6) SAr: *S. arenarium*, (7) SR: *S. resinosum*,
 (8) SC: *S. corymbosum*, (9) SCa: *S. campestre*, (10) SP: *S. petraeum*

Table 3. Inhibitory effects of *Seseli* extracts on DPPH stable radical

Samples	AcOEt Extracts	MeOH Extracts
	IC ₅₀ (mg/mL)	IC ₅₀ (mg/mL)
Control		
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±0.05
SL	0.75±0.07	0.187±0.002
SGG	3.07±0.04	0.088±0.001
SAr	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	

^aEach value represents the mean ± S.D. of 2-4 independent experiments

^bEtOAc and MeOH only, control for extracts.

^cNE: Not effect

SA: *S. andronakii*, SH: *S. hartvigii*, ST: *S. tortuosum*, SL: *S. libanotis*, SGG: *S. gummiferum* subsp. *gummiferum*, SAr: *S. arenarium*, SR: *S. resinosum*, SC: *S. corymbosum*, SCa: *S. campestre*, SP: *S. petraeum*

Table 4. Anti-lipid peroxidation effects (LPO) of <i>Seseli</i> extracts ^a						
	Concentrations mg/mL	Nmol MDA/g tissue	% Inhibition	Concentrations mg/mL	nmol MDA/g tissue	% Inhibition
Control	AcOEt Extracts			MeOH Extracts		
^b			NE ^c			NE ^c
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
SAr	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	0.22	0.009	96
	0.44	0.003	99	0.44	0.003	99

^aEach value represents the mean ± S.D. of 2–4 independent experiments

^bEtOAc or MeOH only, control for extracts.

^cNE: Not effect

SA: *S. andronakii*, SH: *S. hartvigii*, ST: *S. tortuosum*, SL: *S. libanotis*, SGG: *S. gummiferum* subsp. *gummiferum*, SAr: *S. arenarium*, SR: *S. resinosum*, SC: *S. corymbosum*, SCa: *S. campestre*, SP: *S. petraeum*