Original Article

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Investigation of the Chemical Composition and Antimicrobial Activity of Endemic *Seseli salsugineum* A. Duran and Lyskov Essential Oil

Endemik *Seseli salsugineum* A. Duran ve Lyskov Uçucu Yağının Kimyasal Bileşimi ve Antimikrobiyal Aktivitesinin Araştırılması

Gözde ÖZTÜRK[1], Betül DEMİRCİ[1], Gamze GÖGER[2], Ahmet DURAN[3], Kemal Hüsnü Can BAŞER[4]

[1]Anadolu University Faculty of Pharmacy, Department of Pharmacognosy, Eskişehir, Türkiye
[2]Afyonkarahisar Health Sciences University Faculty of Pharmacy, Department of Pharmacognosy, Afyonkarahisar, Türkiye
[3]Selçuk University Faculty of Science, Department of Biology, Konya, Türkiye
[4]Near East University Faculty of Pharmacy, Department of Pharmacognosy, Nicosia, Cyprus

ABSTRACT

Objective: The main purpose of this study was to determine the chemical compositions and antimicrobial activity of the essential oil from the aerial parts of the recently discovered endemic species *Seseli salsugineum (S. salsugineum)* A. Duran and Lyskov for the first time.

Methods: Essential oil from the aerial parts of *S. salsugineum* was isolated by using a Clevenger-type apparatus and the oil was analyzed by gas chromatography (GC) and GC-mass spectrometry (GC-MS), simultaneously. Furthermore, antimicrobial activity of the essential oil was tested against Gram-negative (*Pseudomonas aeruginosa* ATCC B888), Gram-positive (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* NRRL B-3711), and three fungal strains: *Candida albicans* ATCC 24433, *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 by broth microdilution method. Chloramphenicol and amphotericin B were used as positive controls. Minimum inhibitory concentrations were determined.

Results: Dried aerial parts of *S. salsugineum* yielded 0.28% (v/w) essential oil. GC and GC-MS analyses resulted in the

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Amaç: Yeni keşfedilmiş endemik bir tür olan *Seseli salsugineum (S. salsugineum)* A. Duran and Lyskov'un toprak üstü kısımlarından elde edilen uçucu yağın kimyasal bileşimi ve antimikrobiyal aktivitesinin ilk kez incelenmesi amaçlanmıştır.

Yöntemler: *S. salsugineum*'un toprak üstü kısımlarının Clevenger apareyi ile uçucu yağı elde edilmiş ve uçucu yağın bileşimi gaz kromatografisi (GK) ve GK-kütle spektrometresi (GK-KS) ile belirlenmiştir. Uçucu yağın antimikrobiyal aktivitesi mikrodilüsyon yöntemi ile Gram-negatif (*Pseudomonas aeruginosa* ATCC B888), Gram-pozitif (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* NRRL B-3711), ve üç fungus suşu: *Candida albicans* ATCC 24433, *Candida parapsilosis* ATCC 22019 ve *Candida krusei* ATCC 6258 suşlarına karşı çalışılmıştır. Pozitif kontrol olarak kloramfenikol ve amfoterisin B kullanılmış ve minimum inhibisyon konsantrasyonları belirlenmiştir.

Bulgular: *S. salsugineum* uçucu yağı verimi %0,28'dir (h/a). GK ve GK-KS analizinde uçucu yağın ana bileşenler olarak sabinen (%35,5), kessan (%10,5), α-pinen (%6,4) ve terpinen-4-ol (%5,0)

Address for Correspondence: Gözde Öztürk, Anadolu University Faculty of Pharmacy, Department of Pharmacognosy, Eskişehir, Türkiye
E-mail: g.ozturkau@gmail.com **ORCID ID:** orcid.org/0000-0002-3998-8859

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ABSTRACT

characterization of sabinene (35.5%), kessane (10.5%), α-pinene (6.4%), terpinen-4-ol (5.0%) as main constituents. The essential oil was found to be effective against all tested strains (320-1280 µg/mL).

Conclusion: To the best of our knowledge, this study is the first report on the chemistry and biological activity of *S. salsugineum* essential oil.

Keywords: *Seseli salsugineum*, Apiaceae, essential oil, antibacterial activity, antifungal activity

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bulunmuştur. Uçucu yağ test edilen tüm suşlara karşı (320-1280 µg/mL) antimikrobiyal aktivite göstermiştir.

Sonuç: Bildiğimiz kadarıyla, bu çalışma ile *S. salsugineum* uçucu yağının kimyası ve biyolojik aktivitesi ilk kez çalışılmıştır.

Anahtar Sözcükler: *Seseli salsugineum*, Apiaceae, uçucu yağ, antibakteriyel aktivite, antifungal aktivite

Introduction

The genus *Seseli* (Apiaceae) is represented by about 125-140 taxa in the world. It is widespread in Euro-Siberian and Eastern Mediterranean regions including Türkiye (1). The Flora of Türkiye comprises 13 taxa in Türkiye, including 8 endemic species (1-3). Recently a new *Seseli* species is identified. The newly described species, *Seseli salsugineum (S. salsugineum)* A. Duran and Lyskov is a narrow endemic species, confined to Lake Tuz area (Konya province) in Central Anatolia, Türkiye. It grows in salt marshes and salt steppes. It is an element of the Irano-Turanian phytogeographic region (4). Morphological features of *S. salsugineum* is described by some researchers (4-6).

This genus contains essential oil, coumarines, terpenoids and polyacetylenes (7). Essential oils and coumraines are major secondary metabolites of this genus and these may be responsible for their bioactivities (3,7). Seselinal, sesibiricol, sibirinol, sesibiricin, isosibiricin, osthol, coumurrayin, sesebrin, sesebrinol, sibiricin, imperatorin, bergapten, xanthotoxin, isopimpinellin and mexoticin were isolated from *Seseli* species (8). Sabinene, α-pinene, β-phellandrene, germacrene D and limonene were identified as major components in essential oils of *Seseli* sp. (9-11). It is widely used in traditional medicine due to its antibacterial, antifungal (3), insect repellent (3,12), emmenagogue, anti-bloating (12), anti-inflammatory (3,13), antinociceptive (13) anti-tumor (3,13-18), anti-rheumatic (19), and antioxidant (20) activities. It has importance due to the essential oil of the genus *Seseli* which is used in traditional medicine and has therapeutic properties especially antimicrobial activity (5).

The present study was designed to elucidate the chemical composition and antimicrobial activity of *S. salsugineum* essential oil.

Methods

Plant Material

Aerial parts of the recently discovered narrow endemic species *S. salsugineum* A. Duran and Lyskov was collected from near Lake Tuz (Türkiye. C4 Konya: Cihanbeyli, between Gölyazı-Lake Tuz, 9th kilometer, 923 m, salty marshes, 25.09.2011, Duran et al. (4) 9855 (Herbarium: HUB). The essential oil was obtained by water distillation for 3 h from 150 g air-dried material, using a Clevenger-type apparatus.

Gas Chromatography and Gas Chromatography Mass Spectrometry Analyses

Gas chromatography (GC) and GC-mass spectrometry (GC-MS) conditions were described previously (21). Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder 4.0 Library), and in-house "Başer Library of Essential Oil Constituents" was built up by genuine compounds and components of known oils (22,23).

Antimicrobial Activity

Bacillus cereus NRRL B-3711 (NRRL-Agricultural Research Service Culture Collection), *Staphylococcus aureus* ATCC BAA 1026 (ATCC-American Type Culture Collection), *Pseudomonas aeruginosa* ATCC B888, *Candida albicans* ATCC 24433, *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258 were used as test microorganisms.

The antimicrobial activity of the essential oil was evaluated by broth microdilution assay according to a modified Clinical and Laboratory Standards Institute method (25). Since this study was an *in vitro* study, ethics committee approval was not required. Mueller Hinton Agar (MHA), Mueller Hinton Broth (MHB) for bacteria, Potato Dextrose Agar (PDA) and Roswell Park Memorial Institute (RPMI) 1640 for fungi were provided and prepared by diluting with distilled water appropriately. Laboratory materials, mediums and contaminated materials used in antimicrobial activity experiments were sterilized in an autoclave at 121 °C under 1.5 atm pressure for 20 minutes. Bacteria were used as MHA medium and PDA medium for *Candida* strains. The prepared media were stored at +4 °C for a maximum of 2 weeks. Their purity was checked, and the microorganisms stored in 15% glycerol solution at -85 °C were inoculated into the prepared media and allowed to multiply by incubating in a bacteriological oven at 37 °C for 24 hours. Turbidity adjustment was made using a turbidimeter according to the developing cultures McFarland No: 0.5 (approximately 10^8 CFU/mL for bacteria) tube (24).

The essential oil (20-0.019 mg/mL) was dissolved in sterile dimethyl sulfoxide for the initial stock solution. One hundred µL of essential oil was applied to -96well microplates and 2 fold serial dilutions were performed. After the dilutions, 50 µL aliquots of turbidimetrically adjusted microorganisms were inoculated on to the plates. After incubation in MHB and RPMI mediums at 37 °C for 24 h, the first well was treated with 20 µL of resazurin, which insured on all microplates the minimum inhibitory concentrations (MIC) where the lowest concentration of the samples prevented visible growth. Solvent and microbial controls were also added to the assay plate. Antimicrobial assays were repeated at least three times for all the test samples. MIC of the samples were determined and compared with both positive controls. The reference drugs, chloramphenicol (for bacteria) and amphotericin B (for fungus) (Sigma-Aldrich) were used as positive controls (24).

Results

The yield of essential oil was obtained by hydrodistillation from the aerial parts of *S. salsugineum* was 0.28% (v/w). It was analysed by GC and GC-MS, simultaneously. A total of 42 compounds were identified in the essential oil of *S. salsugineum*, which represented 93.7% of the oil. Sabinene (35.5%), kessane (10.5%), α-pinene (6.4%) and terpinen-4-ol (5.0%) were characterized as main constituents. Other components are given in Table 1.

Antimicrobial activity of the essential oil (40-2560 µg/mL) compared to reference antibiotics (2-64 µg/mL), was tested against the following bacterial strains: Gram-negative (*Pseudomonas aeruginosa* ATCC B888), Gram-positive (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* NRRL B-3711), and three fungal strains: *Candida albicans* ATCC 24433, *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258; results are presented in Table 2.

The essential oil generally showed the best action (320 µg/mL) against the tested microorganisms except for *P. aeruginosa* strain (1280 µg/mL).

Discussion

In previous works on the other species of *Seseli*, the most abundant compound in the essential oil *S. rigidum* fruit oil was found as monoterpenes α-pinene (37.8%) and sabinene (13.5%) (25). Goncalves et al. (26) reported that α-pinene (24.8-24.9%), β-pinene (23.5-23.9%) and (*Z*)-β-ocimene (13.3-16.0%) were major constituents in the essential oils of *S. tortuosum*. The main constituents of the oils of *S. montanum* subsp. *peixotoanum* were α-pinene (36.0-37.1%), β-pinene (22.5-23.6%) and limonene (7.7-8.8%). (*Z*)-β-Ocimene was a minor component in *S. montanum* subsp. *peixotoanum* oils. Among the sesquiterpenes, β-elemene was the major one (5.2-5.8%) (26). In another study, the essential oils obtained from the different parts of *S. rigidum* (flower, leaf and fruit) were analysed and α-pinene (33.0, 26.3, 33.2%), sabinene (7.9, 7.8, 18.5%) and limonene (7.1, 5.4, 8.7%) were found as major components (27). According to

Table 1. Volatile constituents of essential oil of *Seseli salsugineum*

RRI	Compound	%
1032	α-Pinene	6.4
1035	α-Thujene	0.6
1076	Camphene	0.8
1118	β-Pinene	2.3
1132	Sabinene	35.5
1159	δ-3-Carene	0.3
1174	Myrcene	2.0
1188	α-Terpinene	0.4
1203	Limonene	2.0
1210	2-Methyl-2-butenal	0.1
1218	β-Phellandrene	0.8
1246	(Z)-β-Ocimene	0.4
1255	γ-Terpinene	1.3
1266	(*E*)-β-Ocimene	0.5
1280	*p*-Cymene	3.0
1290	Terpinolene	0.3
1553	Linalool	0.7
1556	*cis*-Sabinene hydrate	0.3
1571	*trans-p*-Menth-2-en-1-ol	0.2
1611	Terpinen-4-ol	5.0
1617	Lavandulyl acetate	0.7
1638	*cis-p*-Menth-2-en-1-ol	0.2
1668	(*Z*)-β-Farnesene	1.0
1704	γ-Muurolene	0.3
1709	α-Terpinyl acetate	0.6
1744	δ-Selinene	1.0
1726	Germacrene D	2.3
1755	Bicyclogermacrene	0.3
1772	Neryl isobutyrate	0.5
1773	δ-Cadinene	1.3
1786	Neryl propionate	0.6
1786	Kessane	10.5
2069	Germacren-D-4-ol	1.5
2144	Spathulenol	1.4
2187	T-Cadinol	0.5
2198	Thymol	0.3
2209	T-Muurolol	1.0
2228	Acorenone B	2.5
2232	α-Bisabolol	0.6
2255	α-Cadinol	2.7
2278	*cis*-Guaie-9-en-11-ol	0.5
2369	Eudesma-4(15),7-dien-1-β-ol	0.5
	Total	93.7

RRI: Relative retention indices calculated against *n*-alkanes, %: calculated from FID data

Table 2. Minimum inhibitory concentrations values (µg/mL)

	S. salsugineum	Chloramphenicol	Amphotericin B
B. cereus	320	4	-
S. aureus	320	8	-
P. aeruginosa	1280	16	-
C. albicans	320	-	8
C. parapsilosis	320	-	64>
C. krusei	320	-	64>

the Tosun et al. (28) the main constituents were determined as germacrene D (54.1%) and sabinene (22.4%) in *S. gummiferum* and *S. corymbosum* subsp. *Corymbosum,* and *β*-phellandrene (29.2%), *α*-phellandrene (8.2%) and germacrene D (2.5%) in *S. corymbosum* subsp. *corymbosum* essential oil.

According to a previous study, evaluation of MIC of the oils showed variability of inhibition among all the fungi tested, *Candida albicans* ATCC 10231, *C. tropicalis* ATCC 13803, and *C. parapsilosis* ATCC 90018. *S. rigidum* oil proved to be more active with MIC and MLC values ranging from 0.64 to 1.25 µL/mL (27). In another work, the antimicrobial activity of essential oils from *S. rigidum* (root, leaf, flower and fruit) were tested against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 10876, *Candida albicans* ATCC 16404 and *Aspergillus niger* ATCC 10231. MIC values were found ranging from 0.02 to 3.24 mg/mL (26).

Conclusion

The *Seseli salsugineum* sp. was discovered and published by Duran et al.'s study in 2021. The essential oil was obtained and its antimicrobial activity was demonstrated. Compared with the literature, to the best of our knowledge, this is the first report on the chemical constituents and antimicrobial activities of the *S. salsugineum* essential oil.

Ethics

Ethics Committee Approval: Ethics committee approval is not required.

Informed Consent: Informed consent is not required.

Footnotes

Authorship Contributions

Concept: G.Ö., B.D., A.D., K.H.C.B., Design: G.Ö., B.D., A.D., K.H.C.B., Data Collection or Processing: G.Ö., G.G., Analysis or Interpretation: G.Ö., B.D., G.G., Literature Search: G.Ö., G.G., Writing: G.Ö., B.D., G.G., A.D., K.H.C.B.

Conflict of Interest: No conflict of interest was declared by the authors.

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