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Research Article

Investigation of the essential oil composition and biological activities of the essential oil and extracts of the aerial parts of *Seseli libanotis* W.D.J. Koch

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ABSTRACT

In this study, the essential oil and various extracts (*n*-hexane, ethyl acetate, and methanol) were obtained from *Seseli libanotis* W.D.J. Koch aerial parts. The essential oil was obtained via hydrodistillation and subjected to GC and GC/MS analysis. 42 compounds were identified as a major component of acorenone B (43.3%), following by *cis*-sesquisabinene hydrate (9.3%), and *trans*-sesquisabinene hydrate (7.7%). The antioxidant activities of the essential oil and extracts were evaluated using DPPH$^{\bullet}$ and TEAC assays. While the methanol extract exhibited the highest antioxidant activity, no significant tyrosinase inhibition was observed.

Keywords: Antioxidant activity, *Seseli libanotis*, tyrosinase inhibition, volatile constituents

1. INTRODUCTION

The *Seseli* genus belongs to the Apiaceae family and represented in the Flora of Turkey by 10 species. *Seseli libanotis* known as 'moon carrot' or 'mountain stone-parsley' is common in the eastern area of Türkiye. Herb used as a aroma source and preservative in cheese [1,2] and its leaves are consumed as vegetable in eastern Türkiye [3].

Characteristic aroma are associated with its volatile constituents and a few studies have been reported on the composition of essential oils from different parts of plant [1,4-6]. Previous studies has been demonstrated the various biological properties of *S. libanotis* such as antioxidant [7,8], antimicrobial [4-5,8], antiinflamatory [3] activity.

Natural products have long been a significant source of bioactive compounds, serving as an inspiration for drug discovery and development. Among these, essential oils and plant extracts have gained attention due to their distinct chemical compositions and potential pharmacological activities.

In the present study, the essential oil obtained from the aerial parts of *S. libanotis* was analyzed by GC-FID and GC/MS to identify its chemical composition. Additionally, sequential extracts were prepared using hexane, ethyl acetate, and methanol. Antioxidant (DPPH radical scavenging and TEAC) and anti-tyrosinase activities of EO and extracts were evaluated. Furthermore, total phenolic and total flavonoid contents of extracts were determined.

2. MATERIALS AND METHODS

2.1. Plant Material

The aerial parts of *Seseli libanotis* were collected from Artvin, Hatila Valley (forest clearing, 1965 m altitude, 41°07.53'N, 45°35.4'E) on August 18, 2014 (Ahmet Duran, 10027).

2.2. Chemicals and Reagents

All chemicals and solvents were of high purity and at least of analytical grade and purchased from Sigma-Aldrich or Merck.

2.3. Isolation of Essential Oil

The essential oil obtained by hydrodistillation, using a Clevenger-type apparatus for 3h and stored at +4°C in the dark until the analysis.

2.4. Extraction

The extracts were prepared using the maceration method with sequential extraction employing solvents of increasing polarity (*n*-hexane, ethyl acetate, and methanol). Each extraction step was performed in triplicate using an automatic shaker at 150 rpm under room temperature and in darkness for 24 hours. The extracts were concentrated to dryness using a rotary evaporator (<40 °C), and their yields were calculated as 3.6%, 3.5%, and 8.9% for hexane, ethyl acetate, and methanol extracts, respectively.

2.5. GC and GC/MS Analysis

An Agilent 6890N GC system (Agilent, USA; SEM Ltd., Istanbul, Türkiye) was used for GC studies. FID temperature was adjusted to 300°C and the same operating conditions were applied to the same column used in GC/MS analysis. Simultaneous auto injection was employed to obtain equivalent retention times. Relative percentages were determined from integration of the peak areas in the chromatograms.

An Agilent 5975 GC-MSD system was used to perform GC/MS analysis. Chromatographic separation was perfomed by using Innowax FSC column (60m x 0.25mm, 0.25µm film thickness) with helium as carrier gas (0.8 mL/min.). Temperature of oven was adjusted at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. The interphase temperature was at 280°C. Split ratio was 40:1 and the injector temperature was set to 250°C. MS were taken at 70 eV between the mass range *m/z* 35 to 450.

2.6. Identification of Compounds

Mass spectra of the components were compared with in-house Baser Library of Essential Oil Constituents, Adams Library [9], MassFinder Library [10], Wiley GC/MS Library [11], and determined by retention indices. These identifications were obtained by comparing their relative retention index (RRI) to a set of *n*-alkanes or retention times with authentic samples [12]. FID chromatograms were used to determine the relative percentage quantities of the separated constituents.

2.7. Antioxidant Activities

The antioxidant activities of the samples were assessed using the DPPH$^{\bullet}$ scavenging and Trolox equivalent antioxidant capacity (TEAC) methods. For DPPH$^{\bullet}$ activity, the method described by Ağalar and Temiz (2021) was employed [13]. Briefly, 8-fold serially diluted samples were incubated with 0.2 mM DPPH$^{\bullet}$ solution for 30 minutes in the dark, and absorbance was recorded at 517 nm. Gallic acid was used as a positive control.

The TEAC assay, following the method of Re et al. (1999), utilized ABTS+$^{\bullet}$ radicals generated by mixing 7 mM ABTS+$^{\bullet}$ and 2.5 mM potassium persulfate, which were left to react for 16 hours in the dark [14]. Samples were mixed with ABTS+$^{\bullet}$, and absorbance was measured at 734 nm after 30 minutes. Results were expressed as Trolox equivalent antioxidant capacity (mmol/L Trolox).

2.8. Total Phenolic and Total Flavonoid Content

Folin-Ciocalteu method was employed to determine the phenolic content [15]. The calibration curve ($y=0.853x + 0.0988$, $R^2 = 0.9994$) was prepared from various concentrations of gallic acid (1-0.8-0.6-0.4-0.2-0.1 mg/mL) and results were expressed as mg gallic acid equivalent (GAE).

$AlCl_3$ method was used to measure the flavonoid content and the absorbances were recorded at 410 nm [16]. Different concentration of quercetin (1-0.8-0.6-0.4-0.2-0.1 mg/mL) ($y = 1.7604x + 0.0251$, $R^2 = 0.9983$) was prepared to create the calibration. Results were calculated as mg quercetin equivalent (QE).

2.9. Tyrosinase Inhibition

The tyrosinase inhibitory activity of the EO and extracts was evaluated by using L-DOPA [17]. Enzyme (200U/mL) and substrate (5 mM) were dissolved in the 0.1 M phosphate buffer (pH 6.8). Briefly, 20 µL sample and 20 µL enzyme was incubated at 25 ˚C for 10 min. Then, reaction was initiated by adding 160 µL L-DOPA and incubated at 25 ˚C for 10 min. Absorbance at 475 nm of each well was measured and kojic acid used as positive control.

2.10. Statistical Analysis

All the experiments were permormed in triplicate, and data were expressed as means ± standard deviation (SD) by using Sigmaplot 14.0 software (Systat Software, Inc., San Jose, CA, USA). IC_{50} values were calculated by regression analysis.

3. RESULTS AND DISCUSSION

3.1. Chemical Composition of *Seseli libanotis* EO

The EO of *Seseli libanotis* was analysed by GC-FID, and GC/MS systems, simultaneously. The essential oil yield was calculated as 0.48% (*v/w*). Fourty-two constituents were determined as representing 92 % of the EO. The most abundant compound was acorenone B (43.3%), making it the major component of the oil. In the literature, aceorenone B has been identified as a major component of the essential oils of plants such as *Niphogeton dissecta* (Benth) J.F. Macbr, *Euphorbia maccorrhiza*, and *Acorus calamus* L., and it has been particularly associated with cholinesterase inhibition properties [18-20]. Other significant constituents included *cis*-sesquisabinene hydrate (9.3%) and *trans*-sesquisabinene hydrate (7.7%), both of which are oxygenated sesquiterpenes and contribute to the chemical complexity of the oil. The oil also contained spathulenol (2.7%) and humulene epoxide-II (2.3%), along with smaller amounts of caryophyllene oxide (1.9%) and carotol (1.2%), indicating a high prevalence of oxygenated sesquiterpenes. Monoterpenes were present in lower concentrations, with α-pinene (0.5%) and limonene (0.2%) being the most prominent compounds (Table 1).

The essential oil composition of *Seseli libanotis* varies considerably depending on the plant part and extraction method used. Skalicka-Wozniak et al. (2010) analyzed the essential oil from fruits using hydrodistillation and HS-SPME, identifying sabinene and *β*-phellandrene as the dominant compounds, particularly in HS-SPME extracts where sabinene reached 46.2% [5]. Furthermore, Masoudi et al. (2006) reported the acorenone (35.5%) as the major volatile substance alongside limonene and α-pinene [6]. Ozturk and Ercisli (2006) found that *trans*-caryophyllene, spathulenol, and caryophyllene oxide was the major components of the aerial parts [4]. The study of Chizzola et al. (2019) revealed that the germacrene D was prevalent in leaves and stems, while fruits were conteined higher amounts of *β*-phellandrene and acorenone B [1]. Additionally, their root volatiles indicated a dominance of *α*-pinene. In the present study, acorenone B was identified as the major component of aerial parts. These differences could associated with chemodiversity of *S. libanotis*, influenced by plant part and geographic origin.

3.2. Antioxidant Activities of Oil and Extracts

The DPPH radical scavenging assay revealed that the methanol extract with a IC_{50} value of 65.2 µg/mL, indicating the highest radical scavenging activity among the tested samples. The ethyl acetate extract showed an IC_{50} value of 480.3 µg/mL. In contrast, the hexane extract and essential oil exhibited limited activity, with IC_{50} values exceeding 2500 µg/mL and 7500 µg/mL, respectively (Table 2).

The TEAC assay provided similar results, with the methanol extract displaying the highest Trolox equivalent antioxidant capacity (1.51 ± 0.02 mM), followed by the ethyl acetate extract (1.00 ± 0.07 mM) and hexane extract (0.34 ± 0.01 mM). The essential oil did not show significant TEAC activity.

Table 1. Chemical composition of the essential oil of *S. libanotis*

RRI	Compounds	%	IM
1032	α-Pinene	0.5	t_R, MS
1118	β-Pinene	0.1	t_R, MS
1132	Sabinene	0.1	t_R, MS
1159	δ-3-Carene	0.2	t_R, MS
1203	Limonene	0.2	t_R, MS
1218	β-Phellandrene	0.1	t_R, MS
1280	*p*-Cymene	1.0	t_R, MS
1528	α-Bourbonene	tr	MS
1568	*trans*-α-Bergamotene	tr	MS
1589	α-Cedrene	tr	MS
1590	Bornyl acetate	tr	t_R, MS
1591	β-Funebrene (=1,7-diepi-β-cedrene)	0.9	t_R, MS
1600	β-Elemene	2.2	MS
1661	Sesquisabinene	0.6	t_R, MS
1668	*(Z)*-β-Farnesene	0.2	MS
1687	α-Humulene	0.3	t_R, MS
1690	α-Acoradiene	0.2	MS
1694	β-Acoradiene	0.2	MS
1703	γ-Curcumene	0.1	MS
1704	γ-Muurolene	0.2	MS
1726	7-epi-1, 2-Dehydro sesquicineole	1.9	MS
1740	Valensene	0.1	t_R, MS
1741	β-Bisabolene	0.4	t_R, MS
1747	Sesquicineole	0.2	MS
1755	β-Curcumene	0.1	MS
1786	*ar*-Curcumene	2.0	MS
1787	Kessane	0.9	MS
2000	*trans*-Sesquisabinene hydrate	7.7	MS
2008	Caryophyllene oxide	1.9	t_R, MS
2045	Carotol	1.2	MS
2071	Humulene epoxide-II	2.3	MS
2096	*cis*-Sesquisabinene hydrate	9.3	MS
2144	Spathulenol	2.7	t_R, MS
2162	α-Acorenol	1.8	MS
2200	*trans*-Methyl isoeugenol	3.1	MS
2228	Acorenone B	43.3	MS
2232	α-Bisabolol	0.4	t_R, MS
2273	Selina-11-en-4α-ol	0.6	MS

RRI: Relative retention indices calculated against n-alkanes; %: calculated from the FID chromatograms; tr:Trace (<0.1 %). Identification method (IM): tR, identification based on the retention times of genuine compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the in-house Baser Library of Essential Oil Constituents, Adams, MassFinder and Wiley libraries and comparison with literature data.

Table 1. Continued

RRI	Compounds	%	IM
2404	*trans*-Isoelemicine	1.0	MS
2415	Veratr-aldehyde (=3,4-dimethoxy benzaldehyde) (= Methyl vanillin)	1.6	MS
2471	Veratryl acetone (= 3,4-dimethoxy phenyl acetone)	0.9	MS
2931	Hexadecanoic acid	1.5	MS
	Grouped compounds (%)		
	Monoterpene hydrocarbons	2.2	
	Oxygenated monoterpenes	0	
	Sesquiterpene hydrocarbons	7.5	
	Oxygenated sesquiterpenes	73.3	
	Others	9	
	TOTAL %	**92**	

RRI: Relative retention indices calculated against n-alkanes; %: calculated from the FID chromatograms; tr:Trace (<0.1 %). Identification method (IM): tR, identification based on the retention times of genuine compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the in-house Baser Library of Essential Oil Constituents, Adams, MassFinder and Wiley libraries and comparison with literature data.

Table 2. Biological activity properties of *S. libanotis* EO and extracts

	TPC ($mg_{GAE}/g_{extract}$)	TFC ($mg_{QE}/g_{extract}$)	DPPH$^{\cdot}$ (IC_{50}, µg/mL)	TEAC ($mM_{eq.}/g_{ext.}$)	Tyrosinase inhibition %
Hexane ext	22.7 ± 1.4	nd	> 2500	0.34 ± 0.01	nd
EtOAc ext	36.6 ± 0.3	1.1 ± 0.05	480.3 ± 5.1	1 ± 0.07	nd
MeOH ext	72.4 ± 1.03	5.7 ± 0.2	65.2 ± 0.6	1.51 ± 0.02	nd
EO	-	-	> 7500	nd	nd
Gallic acid[c]			1.93 ± 0.02		
Kojic acid[c]					14.28 ± 0.6

Data was given as mean ± SD (*n = 3*). nd: not detected; -: not performed; [c]: Positive controls, values represented IC_{50}, µg/mL).

These results corroborate the DPPH findings, further emphasizing the superior antioxidant capacity of the methanol extract (Table 2).

The antioxidant activities of *S. libanotis* extracts have been extensively evaluated. The methanol extract of the aerial parts demonstrated strong DPPH radical scavenging activity with an IC_{50} of 0.187 mg/mL, while the ethyl acetate extract exhibited moderate activity with an IC_{50} of 0.75 mg/mL [7]. In another study, the methanol extract of *S. libanotis* aerial parts showed notable antioxidant properties with an IC_{50} of 0.46 mg/mL in the DPPH$^{\cdot}$ and ABTS scavenging activity ranging from 1.98 to 2.06 $mg_{VitCequivalent}/g$ [8].

3.3. Total phenolic and flavonoid content

Methanol extract was found to be highest phenolic (72.4 ± 1.03 $mg_{GAE}/g_{extract}$) and flavonoid (5.7 ± 0.2 $mg_{QE}/g_{extract}$) content followed by the ethyl acetate extract with a value of 36.6 $mg_{GAE}/g_{extract}$ and 1.1 ± 0.05 $mg_{QE}/g_{extract}$, respectively. The hexane extract presented the lowest phenolic content (22.7 $mg_{GAE}/g_{extract}$), while flavonoid content was not observed (Table 2). In the study reported by Matejić et al. (2012), methanol extracts of *Seseli libanotis* was studied in terms of phenolic and flavonoid content and results were determined as 85.03 $mg_{GAE}/g_{extract}$, and 12.42 $mg_{QE}/g_{extract}$, respectively [8].

3.4. Tyrosinase inhibiton

Tyrosinase inhibitory properties of extracts and essential oil of *Seseli libanotis* aerial parts were studied for the first time. However, there was no significant activity in tested concentration of samples (Table 2). Therefore, the components of *S. libanotis* may not interact effectively with tyrosinase or lack the necessary chemical features for enzyme inhibition.

4. CONCLUSION

This study provides the comprehensive investigation into the essential oil composition and biological activities of *Seseli libanotis* W.D.J. Koch aerial parts. GC and GC/MS analysis revealed that the acerenone B were the major component of volatile constituents and the sesquiterpenes were exhibited highest content of essential oil. Extracts and the essential oil were evaluated for their antioxidant activities and the methanol extract exhibited the strongest antioxidant properties for both DPPH radical scavenging and TEAC assays. Furthermore, this was the first study on *S. libanotis* EO and extracts for tyrosinase inbition even though no significant effect was observed. Results suggest that the constituents may lack the necessary structural characteristics for tyrosinase interaction. These findings contribute valuable chemical and biological insights into *S. libanotis*, offering a basis for further research into its potential applications in pharmaceutical, cosmetic, and industrial fields.

Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Conceptualization: B.T., M.K., H.G.A.; Methodology: B.T., H.G.A., M.K.; Supervision: K.H.C.B.; Materials: A.D.; Data Collection and/or Processing: M.K., B.T.; Analysis and/or Interpretation: M.K., B.T.; Literature Search: B.T., M.K., H.G.A.; Writing—original draft preparation: B.T., M.K., H.G.A., K.H.C.B.; Critical Reviews: K.H.C.B. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declared that there is no conflict of interest.

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