Original Article

DOI: 10.14235/bas.galenos.2022.37929
Bezmialem Science 2022;10(6):722-34

Anticholinesterase and Anti-inflammatory Activities of Essential Oils of Naturally Grown *Daucus* L. Species in Turkey

Türkiye'de Doğal Olarak Yetişen *Daucus* L. Türlerinin Uçucu Yağlarının Antikolinesteraz ve Anti-enflamatuvar Aktiviteleri

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ABSTRACT

Objective: This present study was conducted to determine the interspecific chemical variability and evaluate the biological effects of the essential oils of *Daucus* L. species growing naturally in Turkey. The species were *D. carota*, *D. broteri*, *D. guttatus*, *D. littoralis*, *D. involucratus* and *D. conchitae* (endemic).

Methods: The essential oils were obtained from fruit samples by distillation method and were analyzed both by GC-FID and GC-MS. The anti-inflammatory and anticholinesterase effects of the essential oils were investigated. The anti-inflammatory effect was evaluated by *in vitro* LOX enzyme inhibition activity. The anticholinesterase effect was tested on AChE and BChE enzymes.

Results: The components, ratios, and yields of *D. carota* essential oils differed depending on the locations where the samples were collected. The main components were detected as carotol (1-74.6%), β-bisabolen (0.9-62.4%), 11αH-himachal-4-en-1β-ol (0.3-49.4%), *trans*-methylisoeugenol (1-45.7%). The main volatile compounds were found in *D. broteri, D. guttatus, D. involucratus, D. littoralis, D. conchitae* as β-sinensal (30.4%), methyleugenol (30.5%), methyleugenol (40.9%), α-humulene (29.4%), methyleugenol (29.6%) respectively. The essential oils didn't exhibit anti-inflammatory activity. Two essential oil samples of *D. carota* showed high anticholinesterase effects compared to the standard. The AChE IC_{50} was calculated as 6.04±0.30 μg/mL, 2.15±0.10 μg/mL (Galantamine IC_{50} 1.13±0.02 μg/mL) and BChE

ÖZ

Amaç: Bu çalışma, Türkiye'de doğal olarak yetişen *Daucus* L. türlerinin uçucu yağlarının türler arası kimyasal değişkenliğini belirlemek ve biyolojik etkilerini değerlendirmek amacıyla yapılmıştır. Türler şunlardır: *D. carota*, *D. broteri*, *D. guttatus*, *D. littoralis*, *D. involucratus* ve *D. conchitae* (endemik).

Yöntemler: Meyve örneklerinden distilasyon yöntemiyle elde edilen uçucu yağlar GK-AİD ve GK-KS sistemleri ile analiz edilmiştir. Uçucu yağların anti-enflamatuvar ve antikolinesteraz etkileri araştırılmıştır. Anti-enflamatuvar etki, *in vitro* LOX enzim inhibisyon aktivitesi ile değerlendirilmiştir. Antikolinesteraz etkisi AChE ve BChE enzimleri üzerinde test edilmiştir.

Bulgular: *D. carota* uçucu yağlarının bileşenleri, oranları ve verimleri, örneklerin toplandığı yerlere bağlı olarak farklılık göstermiştir. Ana bileşenler; karotol (%1-74,6), β-bisabolen (%0,9-62,4), 11αH-himakal-4-en-1β-ol (%0,3-49,4), trans-metilizoöjenol (%1-45,7) olarak tespit edilmiştir. *D. broteri, D. guttatus, D. involucratus, D. littoralis, D. conchitae* türlerine ait uçucu yağ ana bileşenleri sırasıyla; β-sinensal (%30,4), metilöjenol (%30,5), metilöjenol (%40,9), α-humulen (%29,4) ve metilöjenol (%29,6) olarak belirlenmiştir. Uçucu yağlar, anti-inflamatuvar aktivite göstermemiştir. *D. carota* türüne ait iki uçucu yağ numunesi, standarda kıyasla yüksek antikolinesteraz etki göstermiştir. AChE IC_{50} 6,04±0,30 μg/mL, 2,15±0,10 μg/mL (Galantamin IC_{50} 1,13±0,02 μg/mL) ve BChE IC_{50} 11,32±0,20 μg/mL, 31,03±0,02 μg/mL (Galantamin

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Cite this article as: Büyükkılıç Altınbaşak B, Ecevit Genç G, Zengin Kurt B, Demirci B. Anticholinesterase and Anti-inflammatory Activities of Essential Oils of Naturally Grown *Daucus* L. Species in Turkey. Bezmialem Science 2022;10(6):722-34

Received: 09.12.2021
Accepted: 21.02.2022

IC_{50} 11.32±0.20 μg/mL, 31.03±0.02 μg/mL (Galantamine IC_{50} 12.15±0.36 μg/mL). These essential oils contained high levels of 11αH-himachal-4-en-1β-ol (25.04%, 49.42%).

Conclusion: Because of their anticholinesterase potential, some *D. carote* essential oils can be evaluated in the preparation of pharmaceutical or nutraceutical products as a complementary therapy for Alzheimer's disease by standardizing their components.

Keywords: *Daucus* spp., essential oil, anti-inflammatory, anticholinesterase

IC_{50} 12,15±0,36 μg/mL) olarak hesaplanmıştır. Bu uçucu yağlar yüksek seviyelerde 11αH-himachal-4-en-1β-ol (%25,04, %49,42) içermektedir.

Sonuç: Antikolinesteraz potansiyelleri nedeniyle bazı *D. carota* uçucu yağları, bileşenleri standardize edilerek Alzheimer hastalığında tedaviye destek amaçlı farmasötik veya nutrasötik ürünlerin hazırlanmasında değerlendirilebilir.

Anahtar Sözcükler: *Daucus* spp., uçucu yağ, anti-enflamatuvar, antikolinesteraz

Introduction

Research on plants for protection of health and treatment of diseases has come for ages and increasingly continues. Wild plants are an important resource for new drug discoveries. Essential oils are mixtures of natural terpenes with a wide range of pharmacological activities and their preparation from various plant species has become increasingly popular in recent years. Antimicrobial, sedative, antispasmodic, anthelmintic, anti-inflammatory, expectorant and diuretic effects are some of them (1). *Daucus* L. belongs to the essential oil content rich Apiaceae family. In the Flora of Turkey and the East Islands, 6 species including *D. carota* L., *D. broteri* Ten., *D. guttatus* Sibth Sm., *D. littoralis* Sm., *D. involucratus* Sm. and *D. conchitae* W Greuter (endemic) are registered (2,3). *D. carota* L. (carrot) is the best-known species of the genus and is cultured widely around the world. Carrot root is used as food and essential oil derived from fruits is used in perfumery (4). *D. carota* fruits are used in folk medicine as stomachic, carminative, diuretic, anthelmintic, emmenagogue, contraceptive and aphrodisiac. Young aerial parts of wild types are also eaten as vegetables (5).

Phytochemical and biological activity studies on *Daucus* species other than *D. carota* are very limited. Several studies have described that essential oils and extracts obtained from *D. carota* show a wide range of biological activities, such as antifungal, antibacterial, antioxidant, anti-inflammatory, hepatoprotective, antihyperlipidemic and antitumour activities against human oesophageal cancer cell (6-14). *D. guttatus* essential oil showed significant antibacterial activity against *Corynebacterium pyogenes* (15). When examined for use in the treatment of tuberculosis, the essential oil obtained from the aerial parts of *D. littoralis* showed antibacterial effect against *Mycobacterium tuberculosis* (16).

In this paper, the chemical compositions of the fruit essential oils of all *Daucus* species grown in Turkey were comparatively analyzed for the first time. The volatile compounds, extracted using hydrodistillation, were analyzed by gas chromatography/flame ionization detection (GC/FID) and GC-mass spectrometry (GC/MS). Also, *in vitro* anticholinesterase and anti-inflammatory activities of *Daucus* species' essential oils were investigated. .

Examination of plants with ethnomedicinal use in drug research makes important contributions to the development of new drugs. Turkey has a rich floristic structure because experiences different geological periods and covers ecologically different regions. Many plants in Turkey have traditional medicinal uses for various diseases and one of them is *D. carota*. The purpose of this study was to evaluate the biological effects and to determine the interspecific chemical variability of the essential oils of *Daucus* species growing naturally in Turkey. Since *D. carota* is the most common species of the genus, samples taken from different locations were examined and compared. On the other hand, other species of the genus had limited growing areas, so they were collected from only one location. The essential oils, which gave the most effective results in biological activity studies, were divided into sub-fractions to determine the effective components that caused the activity. Content analysis and activity studies of the obtained fractions were performed again.

Methods

Plant Material

The following 6 species belonging to *Daucus* genera were collected by Betül Büyükkılıç-Altınbaşak in the fruiting period from the Marmara, Aegean, and Mediterranean regions: *D. carota*, *D. broteri*, *D. guttatus*, *D. littoralis*, *D. involucratus* and *D. conchitae* (endemic). Plant samples were collected from İstanbul, Denizli, Muğla, and Antalya between June and September in 2016, 2017 and 2018 (Figure 1). Taxonomical determinations of the collected specimens were made using the Flora of Turkey and the Aegean Islands by Dr. Gülay Ecevit-Genç and Betül Büyükkılıç-Altınbaşak (3,17). The voucher specimens were kept at the Herbarium of the Faculty of Pharmacy, İstanbul University (ISTE). Nine samples of *D. carota* species were collected from different locations. Due to the large number of samples examined, a code was given to each sample to avoid confusion. This is presented in Table 1. These scientific names were verified for each of the species using the International Plant Name Index (18).

Extraction of Essential Oils

The dried fruits of *Daucus* species were cut into small pieces and immediately subjected to hydrodistillation in Clevenger apparatus for 3 hours. Essential oils, thus obtained were stored in sealed vials at +4 °C until analyzed and tested.

Gas Chromatography Analysis

The GC analysis was performed on the Agilent 6,890N GC system at an FID detector temperature of 300 °C. To achieve

Table 1. List of *Daucus* species

Scientific name	ISTE herbarium number	Location and collection dates	Code
D. carota	ISTE 115524	A2(A), İstanbul, 119 m, 29.08.2016	D1
D. carota	ISTE 115525	A2(E), İstanbul, 5 m, 30.08.2016	D2
D. carota	ISTE 115526	A2(E), İstanbul, 47 m, 30.08.2016	D3
D. carota	ISTE 115527	A2(E), İstanbul, 119 m, 30.08.2016	D4
D. carota	ISTE 115528	C2, Denizli, 483 m, 10.09.2016	D5
D. carota	ISTE 115529	C2, Denizli, 976 m, 10.09.2016	D6
D. carota	ISTE 115530	C1, Muğla, 50 m, 14.09.2016	D7
D. carota	ISTE 115532	C1, Muğla, 16 m, 15.09.2016	D8
D. carota	ISTE 115533	C2, Denizli, 1158 m, 27.08.2017	D9
D. broteri	ISTE 115534	C2, Denizli, 735 m, 27.08.2017	D10
D. guttatus	ISTE 115531	C1, Muğla, 50 m, 14.09.2016	D11
D. littoralis	ISTE 115790	C2, Antalya, 52 m, 08.06.2018	D12
D. involucratus	ISTE 115792	C3, Antalya, 21 m, 09.06.2018	D13
D. conchitae	ISTE 115791	C2, Antalya, 360 m, 08.06.2018	D14

the same elution order as GC/MS, the simultaneous automatic injection was performed on a replicate of the same column, applying the same operating conditions. The relative percentage amounts of the separated compounds were calculated from the FID chromatograms. The analysis results are given in Table 2.

The GC/MS analysis was performed on an Agilent 5,975 GC/MSD system on an Innowax FSC column (60 m x0.25 mm, 0.25 µm film thickness) using helium (0.8 mL/min) as carrier gas. The GC oven temperature was held at 60 °C for 10 minutes and programmed at 220 °C at 4 °C/min, for 10 minutes, then 240 °C at 1 ratio. °C/min. The division ratio was set to 40:1 and the injector temperature was set to 250 °C. Mass spectra were recorded at 70 eV. The mass range was *m/z* 35 to 450.

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. Commercial (Wiley GC/MS Library, MassFinder Software 4.0) (19,20) and in-house libraries ("Başer Library of Essential Oil Constituents" which was built up by genuine compounds and components of known oils) were used.

Anti-inflammatory Activity

In the present study, the essential oils were evaluated for possible anti-inflammatory activity by *in vitro* soybean lipoxygenase (Soy LOX) (1.13.11.12, Type I-B, 7.9 unit/mg) enzyme inhibition which was performed spectrophotometrically. Based on the study conducted by Baylac and Racine (21) in 2003, the method updated in microscale was applied (21,22). 1.94 mL of potassium phosphate buffer (100 mM; pH: 8.80), 40 µL essential oil samples at a concentration of 100 µg/mL and 20 µL lipoxygenase enzyme were incubated for 10 min at 25 °C. Three hundred µL of this mixture was added to each well of quartz microplate. The reaction was then initiated by the addition of 7.5 µL linoleic acid solution. The experiments were carried out in 3 replicates. The results were calculated by recording the changing absorbance values every minute for 10 minutes in the ELISA reader (ELx808IU) at 234 nm. nordihydroguaiaretic acid (NDGA) was used as a positive control at 20, 12, 4, 3, 2 µg/mL concentrations.

Determination of AChE and BChE Inhibitory Activities

The essential oils' anticholinesterase activity was determined by using *in vitro* AChE and BuChE enzymes inhibition assays. AChE and BuChE inhibition activities were determined using the method found by Ellman et al. (23). Galantamine is used as reference compound. The IC_{50} was determined by constructing an absorbance and/or inhibition (%) curve and examining the effect of seven different concentrations. Acetylthiocholine iodide and butyrylthiocholine iodide were used as substrates in the reaction, and 5,5'-dithio-bis(2-nitrobenzoic) acid (DTNB) was used as a reagent. Stock solutions of essential oils and galantamine were prepared with methanol at a 4,000 µg/mL concentration. Hundred and fifty µL of 100 mM phosphate buffer (pH 8.0), 10 µL of sample solution, and 20 µL of AChE (2.476x10-4 U/µL) (or 3.1813x10-4 U/µL of BuChE) solution were mixed. It was incubated at 25 °C for 15 minutes. Ten µL of DTNB solution at a concentration of 2 mg/mL was added.The reaction was initiated by the addition of 10 µL acetylthiocholine iodide (or butyrylthiocholine iodide). In this method, the activity was measured by following the yellow color produced as a result of the presence of thio anion which was produced by the enzymatic hydrolysis of the substrate with DTNB. Also, methanol was used as a control solvent. The hydrolysis of the substrates was monitored using a BioTek Power Wave XS at 412 nm (24).

Fractionation by Column Chromatography

The essential oils effective in biological activity studies were divided into 2 sub-fractions by column chromatography to increase the proportion of their major components. Silica gel (7733; Merck) was used as column adsorbent in fractionation. It was first eluted with n-hexane and then with ethanol. Accumulated fractions were checked by TLC (thin layer

Table 2. Chemical composition of the essential oils of *Daucus* species

RRI	Compound	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
1032	α-Pinene	0.3	0.2	0.4	0.1	2.3	0.8	0.2	0.4	0.5	2.6	0.1	4.6	2.0	3.0
1035	α-Thujene	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.1
1076	Camphene	-	-	-	tr	0.2	0.1	-	-	tr	0.1	-	0.9	0.1	0.2
1118	β-Pinene	0.2	0.1	0.2	0.1	0.3	0.6	0.1	0.1	0.1	22.3	0.1	0.2	0.1	0.3
1132	Sabinene	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.3	0.2	1.4	0.1	0.2	0.9	1.3
1174	Myrcene	0.1	tr	tr	tr	0.2	0.1	tr	0.1	0.1	1.7	0.1	1.5	0.2	0.4
1188	α-Terpinene	-	-	-	-	-	-	tr	-	-	0.1	-	-	tr	-
1203	Limonene	0.1	0.2	0.1	0.1	-	0.3	0.1	0.2	0.2	0.7	0.1	1.6	0.2	0.7
1218	β-Phellandrene	-	-	-	-	tr	tr	tr	tr	tr	0.3	-	0.5	tr	0.1
1255	γ-Terpinene	tr	-	-	-	-	tr	tr	tr	tr	0.7	tr	0.1	0.2	tr
1266	(E)-β-Ocimene	tr	-	tr	-	-	-	tr	-	-	tr	-	-	0.1	0.2
1290	Terpinolene	-	-	-	-	tr	-	-	-	-	0.1	-	tr	0.1	0.1
1280	p-Cymene	tr	tr	tr	tr	tr	tr	tr	tr	tr	1.7	0.1	0.1	0.4	0.3
1398	3-Octanol	-	-	-	-	-	-	-	-	-	-	-	0.2	-	-
1443	Trans-Sabinene hydrate	-	-	-	-	-	-	-	-	tr	0.1	-	-	0.1	-
1452	1-Octen-3-ol	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-
1466	α-Cubebene	-	-	-	-	-	-	-	-	-	0.4	-	-	0.1	-
1482	Longipinene	1.4	0.3	0.7	-	2.4	-	13.5	5.6	tr	-	-	0.1	-	-
1493	α-Ylangene	-	-	-	-	-	-	0.1	tr	-	-	-	-	-	-
1497	α-Copaene	-	-	-	-	-	-	-	-	-	0.6	-	0.2	0.1	0.2
1504	Daucene	2.1	0.1	-	-	1.8	4.8	-	-	3.3	-	0.4	-	-	-
1513	Longicyclene	-	-	-	-	0.1	-	0.2	0.1	-	-	-	-	-	-
1532	Camphor	-	-	-	-	-	-	-	-	-	-	0.2	7.9	-	0.5
1535	β-Bourbonene	-	-	-	-	-	-	-	-	-	0.2	-	-	tr	-
1549	β-Cubebene	0.4	-	-	-	0.4	1.0	-	-	0.7	1.2	-	0.1	0.3	0.3
1553	Linalool	0.3	0.3	-	-	0.1	0.2	0.3	0.4	0.1	-	0.3	1.9	0.1	0.3
1568	Trans-α-Bergamotene	0.9	0.3	0.4	0.1	0.9	2.0	1.8	2.4	1.2	-	-	-	-	-
1570	Trans-Myrtanal	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-
1583	Junipene (Longifolene)	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-
1586	Pinocarvone	tr	-	-	-	0.1	-	-	-	-	0.6	-	-	-	-
1597	Bornyl acetate	-	-	-	-	-	-	-	-	-	0.2	-	tr	0.1	-
1599	β-Copaene	-	-	-	-	-	0.3	-	-	-	-	-	tr	-	-
1600	β-Elemene	-	-	-	-	-	-	0.1	-	-	-	0.2	0.2	0.1	0.2
1601	Nopinone	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-
1607	Thymol methyl ether	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-
1611	Terpinen-4-ol	-	-	-	-	-	-	-	-	-	0.4	-	-	0.2	-
1612	β-Caryophyllene	0.3	-	0.5	-	0.5	0.6	1.6	1.0	0.6	2.2	10.1	4.4	4.1	3.4
1613	β-Cedrene	-	-	-	-	-	-	-	-	-	-	0.2	-	-	-
1613	Acora-2,4-diene	-	-	-	-	-	-	-	-	-	0.1	-	-	-	0.1
1639	Cadina-3,5-diene	0.2	-	-	-	0.2	0.4	-	-	tr	-	-	-	-	-
1648	Myrtenal	-	-	-	-	-	-	-	-	-	1.0	-	-	-	-
1661	α-Himachalene	0.1	-	-	-	0.1	-	0.6	0.3	-	-	-	-	-	-
1661	Trans-Pinocarvyl acetate	-	-	-	-	-	-	-	-	-	0.2	-	-	-	-
1664	Trans-Pinocarveol	-	-	-	-	0.2	-	-	-	-	0.8	-	-	tr	-
1664	(Z)-β-Santalene	-	-	-	-	-	-	-	-	tr	-	-	-	-	-

Table 2. Continued

RRI	Compound	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
1669	Sesquisabinene	0.1	0.4	0.5	0.4	0.1	-	0.2	0.1	tr	-	-	-	-	-
1668	(Z)-β-Farnesene	1.4	0.2	0.2	0.2	1.1	2.8	0.8	0.5	2.5	0.8	0.2	-	-	-
1674	Sesquisabinene-B	-	-	-	0.2	0.1	-	-	-	tr	-	-	-	-	-
1677	Epizonarene	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4
1683	Trans-Verbenol	0.2	-	0.1	-	0.6	0.1	-	-	-	0.2	0.2	-	0.2	tr
1687	α-Humulene	-	-	0.1	-	0.1	0.1	0.2	0.1	0.4	0.2	1.8	29.4	3.1	8.7
1687	Methyl chavicol (Estragole)	-	-	-	-	-	-	-	-	-	-	-	10.9	-	-
1688	Selina-4,11-diene	-	-	-	-	-	-	-	-	-	-	2.5	0.1	3.8	3.0
1690	α-Acoradiene	-	1.8	-	-	-	-	-	-	-	0.1	-	-	-	-
1693	β-Acoradiene	0.8	-	0.3	1.3	0.2	-	-	-	-	-	-	-	0.1	-
1695	(E)-β-Farnesene	0.1	-	-	-	-	0.2	0.4	0.4	-	-	-	-	-	-
1698	Myrtenyl acetate	-	-	-	-	-	-	-	-	-	0.2	-	-	-	-
1704	γ-Muurolene	-	-	-	-	-	-	-	-	-	-	-	-	0.1	1.0
1706	α-Terpineol	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-
1719	Borneol	-	-	-	-	-	-	-	-	-	-	-	0.3	-	-
1726	Germacrene D	-	-	-	-	-	0.1	-	-	-	0.3	-	5.3	-	1.5
1726	α-Zingiberene	0.1	-	-	-	0.1	0.4	-	-	0.3	-	-	-	-	-
1729	β-Himachalene	0.5	0.1	0.2	-	0.8	-	5.6	2.3	0.1	-	-	-	-	-
1740	α-Muurolene	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1
1741	β-Bisabolene	15.0	47.7	62.4	48.0	5.6	0.9	1.0	8.9	6.0	5.9	-	0.1	1.5	2.3
1742	β-Selinene	-	-	-	-	-	0.4	-	-	-	-	10.4	0.5	10.2	6.5
1743	α-Cadinene	-	-	-	-	-	-	-	-	-	-	0.3	0.1	-	-
1740	α-Selinene	-	-	-	-	-	0.2	-	-	-	-	-	-	-	-
1751	Carvone	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-
1755	Dauca-8,11-diene	0.3	-	-	-	0.5	1.1	-	-	0.9	-	-	-	-	-
1755	Bicyclogermacrene	0.4	-	-	-	-	-	-	-	-	-	-	0.1	-	-
1755	β-Curcumene	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-
1765	Geranyl acetate	-	0.1	-	-	-	-	-	-	-	-	-	-	1.0	0.7
1773	δ-Cadinene	-	-	-	-	-	-	-	-	-	0.3	0.4	0.3	tr	-
1776	γ-Cadinene	-	-	-	-	-	-	-	-	-	-	1.9	0.5	0.2	-
1783	β-Sesquiphellandrene	-	-	-	-	-	0.4	-	-	0.4	-	-	-	-	-
1784	(E)-α-Bisabolene	0.8	1.5	1.8	1.8	0.2	-	0.6	0.5	-	-	-	-	-	-
1785	7-epi-α-Selinene	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1786	ar-Curcumene	-	-	-	-	-	0.1	-	-	0.1	-	-	-	-	-
1804	Myrtenol	-	-	-	-	0.1	-	-	-	-	0.7	-	-	-	-
1854	Germacrene B	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-
1857	Geraniol	2.1	0.1	-	-	-	-	-	-	-	-	-	-	0.6	0.4
1868	Neryl isovalerate	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-
1882	α-Dehydro-ar-himachalene	-	-	-	-	-	-	0.1	tr	-	-	-	-	-	-
1888	ar-Himachalene	0.1	tr	0.3	-	1.0	-	0.6	1.1	-	-	-	-	-	-
1900	epi-Cubebol	-	-	-	-	-	-	-	-	-	0.3	-	-	tr	-
1924	γ-Dehydro-ar-himachalene	-	-	-	-	-	-	0.1	0.1	-	-	-	-	-	-
1957	Cubebol	-	-	-	-	-	-	0.3	0.1	-	0.3	-	-	0.1	-
2001	Isocaryophyllene oxide	-	-	-	-	-	-	-	-	-	0.3	0.9	tr	0.1	0.2
2004	Oxido himachalene	-	-	-	-	-	-	tr	-	-	-	-	-	-	-

Table 2. Continued

RRI	Compound	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
2008	Caryophyllene oxide	0.1	-	0.5	-	0.5	-	0.1	0.2	-	2.1	10.8	0.5	0.7	0.9
2025	Perillyl alcohol	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-
2030	Methyl eugenol	0.2	-	-	-	-	-	tr	0.3	-	-	30.5	0.2	40.9	29.6
2045	Carotol	42.8	1.7	1.9	1.0	51.4	74.6	-	-	63.1	-	5.8	-	-	-
2045	Humulene epoxide I	-	-	-	-	-	-	-	-	-	-	-	0.4	0.2	0.7
2048	6,7-Epoxy-himachalene (β-himachalene oxide)	-	-	-	-	-	-	0.1	0.1	-	-	-	-	-	-
2071	Humulene epoxide II	-	-	-	-	-	-	-	-	-	0.2	1.0	3.0	0.5	3.8
2080	1,10-di-epi-Cubenol	-	-	-	-	-	-	-	-	-	-	0.5	0.2	0.1	0.3
2081	Humulene epoxide III	-	-	-	-	-	-	-	-	-	-	-	0.2	tr	0.1
2088	1-epi-Cubenol	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-
2089	6-Methyl-5-(3-methylphenyl) heptan-2-one	-	-	-	-	-	-	-	-	-	-	0.2	-	-	-
2096	cis-Sesquisabinene hydrate	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-
2104	Viridiflorol	-	-	-	-	-	-	-	-	-	-	0.2	-	-	-
2109	cis-Methylisoeugenol	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-
2131	Hexahydrofarnesyl acetone	-	-	-	-	-	-	-	-	-	0.4	0.3	-	-	-
2131	11αH-himachal-4-en-1β-ol	5.4	1.8	2.7	0.5	11.5	-	49.4	25.0	0.3	-	0.3	-	-	-
2144	Spathulenol	0.2	-	-	-	-	-	-	-	-	0.2	0.2	0.2	-	0.2
2173	6-epi-Cubenol	-	-	-	-	0.5	-	0.8	0.5	-	-	0.4	-	-	-
2187	T-Cadinol	-	-	-	-	-	-	-	-	-	-	8.1	3.5	0.8	6.1
2200	trans-Methylisoeugenol	3.2	5.7	1.0	15.1	-	-	6.6	45.7	-	-	-	0.1	0.1	-
2205	Thymol	-	-	-	-	-	-	-	-	-	-	-	0.3	-	-
2219	2-Himachalen-7-ol	-	-	-	-	-	-	0.5	0.4	-	-	-	-	-	-
2219	Acorenone	-	-	-	0.5	-	-	-	-	-	3.2	-	-	2.9	2.0
2226	Methyl hexadecanoate	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-
2228	Acorenone B	-	-	-	0.5	0.4	-	-	-	-	1.2	-	-	1.0	0.8
2231	Torilenol	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-
2232	α-Bisabolol	0.1	-	-	-	0.6	-	0.3	0.2	0.2	-	-	-	-	-
2237	β-Sinensal	-	-	-	-	-	-	-	-	-	30.4	-	-	-	-
2246	Elemicin	4.8	14.9	1.7	1.9	-	-	-	-	-	-	-	-	2.9	2.1
2248	γ-Asarone	-	-	-	-	-	-	-	-	-	-	-	-	18.5	-
2255	α-Cadinol	-	-	-	-	-	-	-	-	-	-	0.6	0.4	-	0.5
2265	Longiverbenone (Vulgarone B)	-	-	-	-	0.4	-	-	-	-	-	-	-	-	-
2269	Guaia-6,10(14)-diene-4β-ol	-	-	-	-	-	-	-	-	-	-	0.3	-	-	-
2289	cis-Isoelemicine	-	-	-	-	-	-	-	-	-	-	-	-	-	13.7
2296	Myristicin	-	-	-	-	-	-	-	-	0.1	5.7	-	-	-	-
2300	Cryptomerione	0.3	0.7	1.0	0.7	-	-	-	-	-	-	-	-	-	-
2312	Daucol	0.9	-	-	-	4.1	0.4	-	-	0.2	-	-	-	-	-
2316	Caryophylladienol I	-	-	-	-	-	-	-	-	-	0.2	0.4	0.1	0.1	-
2320	Juniper camphor	0.4	-	0.3	-	-	-	-	0.1	0.1	-	-	-	-	-
2324	Caryophylladienol II	-	-	-	-	-	-	-	-	-	0.4	0.7	-	-	-
2349	Cadina-4,10(15)-dien-3-one	-	-	-	-	-	-	-	-	-	-	0.5	-	-	-
2361	β-Asarone	-	1.2	-	-	-	-	-	-	-	-	-	-	-	-
2369	Eudesma-4(15),7-dien-4β-ol	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-
2392	Caryophyllenol II	-	-	-	-	-	-	-	-	-	0.4	1.0	-	-	0.5

Table 2. Continued

RRI	Compound	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
2404	trans-Isoelemicin	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-
2431	Methyl stearate	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-
2467	Methyl elaidate	-	-	-	0.9	-	-	-	-	-	-	-	-	-	-
2478	α-Asarone	2.0	15.2	13.9	18.8	1.1	0.2	0.5	-	-	-	-	-	-	-
2500	Pentacosane	-	-	-	-	-	-	-	-	-	0.2	-	-	-	-
2509	Methyl linoleate	-	-	-	0.9	-	-	-	-	-	-	-	-	-	-
2607	14-Hydroxy-δ-cadinene	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-
2622	Phytol	-	-	-	-	-	-	-	-	-	0.2	-	-	-	-
2670	Tetradecanoic acid	-	-	-	-	-	-	-	-	-	0.3	0.5	-	-	-
2700	Heptacosane	-	-	-	-	-	-	-	-	-	0.3	-	-	-	-
2822	Pentadecanoic acid	-	-	-	-	-	-	-	-	-	0.2	-	-	-	-
2931	Hexadecenoic acid	-	-	0.5	0.5	tr	-	-	-	-	2.8	2.7	0.7	tr	-
	Total	88.8	94.8	91.6	94.4	90.8	93.0	97.1	98.5	83.6	98.0	95.3	82.3	98.7	97.7
	Oil yield %	**1.7**	**2.5**	**1.7**	**1.3**	**1.1**	**2.0**	**2.4**	**1.8**	**1.3**	**0.02**	**0.1**	**0.1**	**0.8**	**0.1**

RRI: Relative retention indices calculated against n-alkanes, % calculated from FID data, and tr: Trace (<0.1%). **D1-9:** *D. carota*, **D10:** *D. broteri*, **D11:** *D. guttatus*, **D12:** *D. littoralis*, **D13:** *D. involucratus,* **D14:** *D. conchitae.*

chromatography) method (Figure 2). The fractions obtained were concentrated by rotavapor. After analyzed with GC/FID and GC/MS systems, biological activity studies were carried out.

Results

Composition of the Essential Oils

The analysis of essential oils obtained by the hydrodistillation method were carried out with GC/FID and GC/MS systems. Comparative analysis results are given in Table 2.

The essential oil samples (D7, D8) belonging to *D. carota* species with high anticholinesterase effects were separated into 2 sub-fractions by column chromatography. As a result of GC/FID and GC/MS analysis, the ratios of the components belonging to *n*-hexane and ethanol fractions are given in Table 3.

LOX inhibition

The essential oils at 100 µg/mL concentration didn't exhibit any anti-inflammatory activity while the positive control, NDGA showed strong anti-inflammatory activity. The IC_{50} value of NDGA was calculated as 9.00±0.01 µg/mL.

AChE and BChE Inhibition

An anticholinesterase effect was observed in three essential oil samples of *D. carota* species. With two of these, we achieved results close to the standard substance galantamine. Essential oils considered to be effective were divided into sub-fractions and cholinesterase inhibition was re-examined using the same method. Calculated IC_{50} values are given in Table 4 and Table 5.

Discussion

The components, ratios, and yields of 9 essential oil samples of *D. carota* species collected from different locations differ according to the regions (Table 2). The main components of the examined *D. carota* essential oils were carotol (1-74.6%), β-bisabolen (0.9-62.4%), 11αH-himachal-4-en-1β-oil (0.3-49.4%) and *trans*-methylisoeugenol (1-45.7%). In previous studies, the main component of the essential oil found in *D. carota* fruits was determined as carotol (66.78%) (25). Also, the main component of essential oil obtained from the aerial parts of *D. littoralis* was determined as *cis*chrysanthenyl acetate (46.8%) (16). In this study, the main component of the essential oil obtained from *D. littoralis* fruits was α-humulen (29.4%). In another study conducted in Turkey, the main components were found as carotol (27.7%), elemicin (18.1%), and limonene (16.0%) in aerial parts of *D. carota* essential oils' (26). The reason for this is thought to be a feature of the Apiaceae family of which members contain different aromatic compounds in their different organs.

The main volatile compound was β-sinensal (30.4%) in *D. broteri,* methyleugenol (30.5%) in *D. guttatus,* methyleugenol (40.9%) in *D. involucratus,* α-humulene (29.4%) in *D. littoralis,* methyleugenol (29.6%) in *D. conchitaeas.* In the literature, the main component of *D. guttatus* fruit essential oil was indicated as β-pinene (18.8%) (27). Such differences are thought to be due to subspecies, because the *Daucus* genus includes systematically problematic species due to its high hybridization rate (28). More studies are needed on its taxonomic status.

The fruit morphology of *Daucus* species is generally similar. Differences in the chemical composition of essential oils support the identification of *Daucus* species.

The chemical composition and biological activity of essential oils can be affected by many factors, such as harvest time and which part of the plant will be used for the essential oil. Significant differences are also found, especially in the composition of *D. carota* fruit essential oils, depending on geographical origin (8).

Our results reinforce previous data on the variability in fruit

Table 3. Chemical composition of *n*-hexane and ethanol fractions of the essential oils

RRI	Compounds	D7-H	D7-E	D8-H	D8-E
1400	Tetradecane	0.4	-	0.5	-
1482	Longipinene	33.5	-	16.1	-
1493	α-Ylangene	0.2	-	tr	-
1513	Longicyclene	0.5	-	0.2	-
1530	β-Longipinene	1.9	-	-	-
1568	Trans-α-Bergamotene	5.4	-	13.1	-
1600	Hexadecane	1.0	-	1.1	-
1610	Calarene	0.2	-	-	-
1612	β-Caryophyllene	3.1	-	2.4	-
1654	1-Hexadecene	0.2	-	-	-
1661	α-Himachalene	1.8	-	-	-
1669	Sesquisabinene	0.2	-	0.4	-
1668	(Z)-β-Farnesene	2.1	-	1.3	-
1687	α-Humulene	0.4	-	0.2	-
1695	(E)-β-Farnesene	1.1	-	2.0	-
1729	β-Himachalene	10.1	-	3.8	-
1741	β-Bisabolene	21.9	-	49.2	-
1744	Eremophylene	5.9	-	1.5	-
1755	β-Curcumene	1.1	-	-	-
1784	(E)-α-bisabolene	0.9	-	1.0	-
1800	Octadecane	1.1	-	1.1	-
1882	α-Dehydroarhimachalene	0.4	-	0.4	-
1888	ar-Himachalene	2.6	-	1.4	-
1924	γ-Dehydroarhimachalene	0.2	-	0.2	-
2000	Eicosane	0.5	-	0.5	-
2008	Caryophyllene oxide	-	0.9	-	0.9
2048	6,7-Epoxy-himachalene (β-Himachaleneoxide)	-	1.2	-	0.7
2131	11αH-himachal-4-en-1β-ol	-	72.2	-	52.0
2173	6-Epicubenol	-	1.4	-	0.8
2200	trans-Methylisoeugenol	-	9.6	-	29.8
2219	2-Himachalen-7-ol	-	-	-	0.8
2232	α-Bisabolol	-	1.0	-	0.5
2300	Tricosane	-	-	0.2	-
2415	Methyl vanillin (veratraldehyde) 3,4-dimethoxybenzaldehyde	-	1.1	-	3.5
2471	Veratrryl acetone (3,4-dimethoxy fenil aceton)	-	-	-	0.8
2478	α-Asarone	-	0.52	-	
	Total	96.9	87.9	96.8	89.8
	Fraction yield %	**41.8**	**58.2**	**33.8**	**66.2**

RRI: Relative retention indices calculated against n-alkanes, % calculated from FID data, and tr: Trace (<0.1%). **D7, D8:** *D. carota* essential oil, **-H:** *n*-Hexane fraction, **-E:** Ethanol fraction
Chromatograms of GC analysis are given in Figures 3-20.

essential oils, depending on the geographical origin of the samples. Differences were observed in essential oils components and ratios of samples collected from different locations. Two samples collected from nearby locations showed strong anticholinesterase activity. Unlike other samples, high levels of 11αH-himachal-4-en-1β-ol (25.04%, 49.42 %) were found in the content of these essential oils.

These essential oils withhighest anticholinesterase activity were divided into sub-fractions to determine the effect of the main component on the activity. The ratios of 11αH-himachal-4-en-1β-ol in the sub-fractions were measured as 72.2% and 52.0%. However, it was observed that the anticholinesterase effect of the fractions decreased. Therefore, the activity is thought to be due to the synergistic effect of the components in the essential oil.

In the literature, it has been reported that the extract prepared with petroleum ether and ethanol from *D. carota* fruits significantly reduces brain acetylcholinesterase activity and cholesterol levels in young and old mice (29). Ethanol extract of *D. carota* seeds has been shown to have a memory-enhancing effect on rats. (30). It has been stated that essential oils can be developed as nutraceuticals in the prevention and improvement of neurodegenerative diseases such as Alzheimer's disease since they have the ability to cross the blood-brain barrier and reach the central nervous system (12). Our findings are in agreement with these results. The essential oil of *D. carota* characterized by high amounts of 11αH-himachal-4-en-1β-ol can be a natural anticholinesterase agent and can be regarded for the management of Alzheimer's disease.

Table 4. AChE and BChE IC50 values of essential oils

Test sample	AChE (µg/mL)	BChE (µg/mL)
D7	6.04±0.30	11.32±0.20
D8	2.15±0.10	31.03±0.02
D9	31.1±0.35	76.8±0.06
Galantamine	1.13±0.02	12.15±0.36

D7-9: *D. carota* essential oil

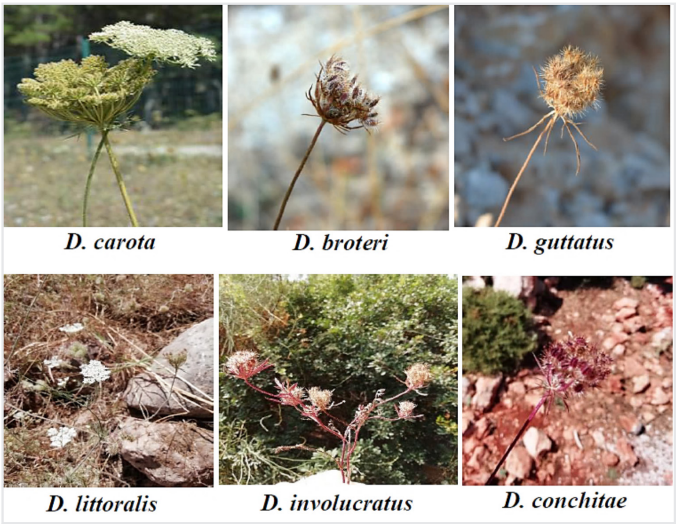

Figure 1. *Daucus* species collected in field studies

Figure 2. Preparation of fractions by column chromatography

Study Limitations

The fact that *D. carota* essential oil compositions and anticholinesterase effects are different increases the possibility that the samples belong to different subspecies. However, there was no taxonomic study on *D. carota* subspecies in Turkey, which limited the chemotaxonomic evaluation of the results. Since different methods were not tested in the anti-inflammatory activity study, the evaluation of this effect was limited.

Table 5. AChE and BChE IC50 values of fractions

Test sample	AChE (µg/mL)	BChE (µg/mL)
D8-H	71.417±0.02	>200
D8-E	181.551±0.30	123.42±0.40
D7-H	132.478±0.14	>200
D7-E	152.267±0.01	111.315±0.56
Galantamine	2.41±0.01	17.38±0.12

>200: IC_{50} value is greater than 200 µg/mL. **-H:** *n*-Hexane fraction of *D. carota* essential oil, **-E:** Ethanol fraction of *D. carota* essential oil

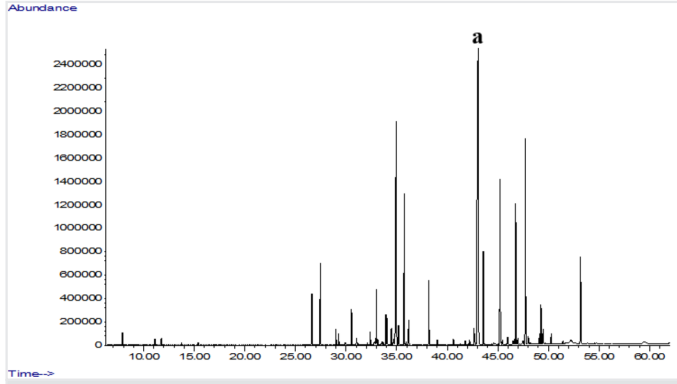

Figure 3. GC chromatogram of essential oil extracted from fruit of *D. carota* (D1) **a:** Carotol (42.8%)

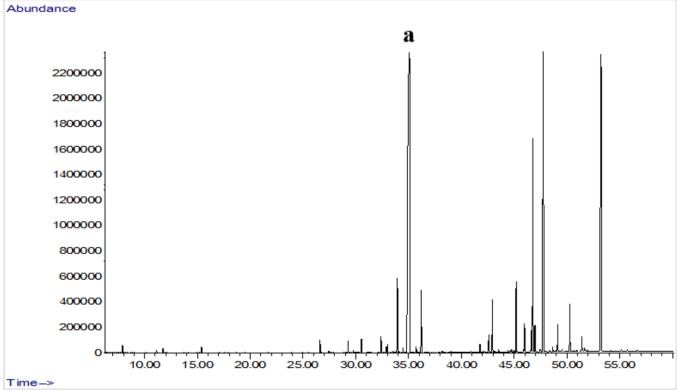

Figure 4. GC chromatogram of essential oil extracted from fruit of *D. carota* (D2) **a:** β-Bisabolene (47.7%)

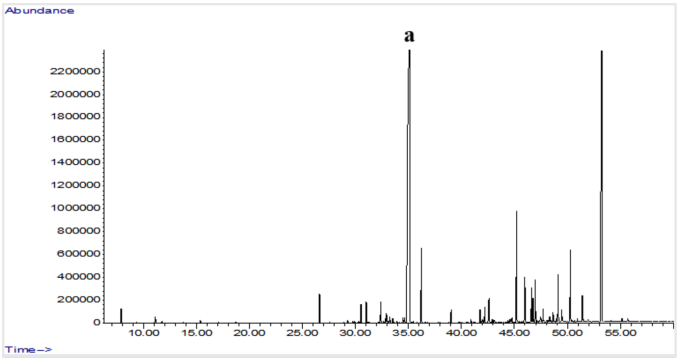

Figure 5. GC chromatogram of essential oil extracted from fruit of *D. carota* (D3) **a:** β-Bisabolene (62.4%)

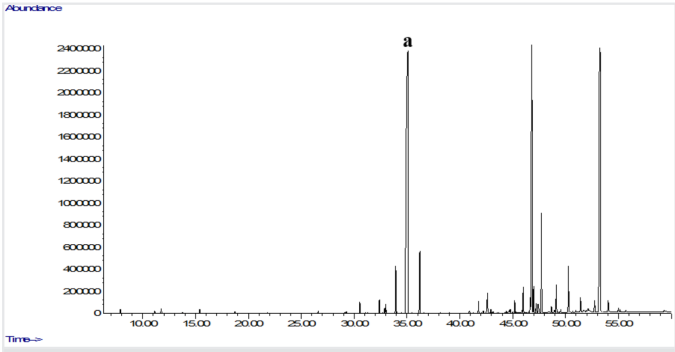

Figure 6. GC chromatogram of essential oil extracted from fruit of *D. carota* (D4) **a:** β-Bisabolene (48.0%)

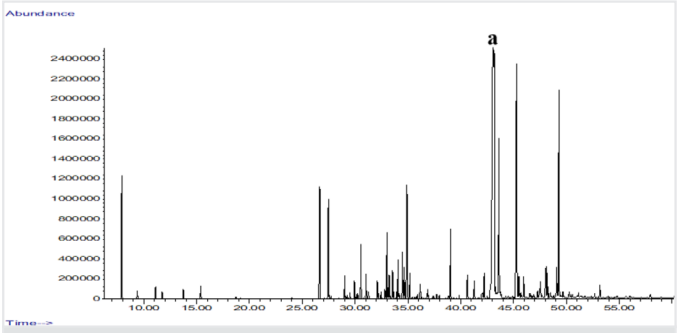

Figure 7. GC chromatogram of essential oil extracted from fruit of *D. carota* (D5) **a:** Carotol (51.4%)

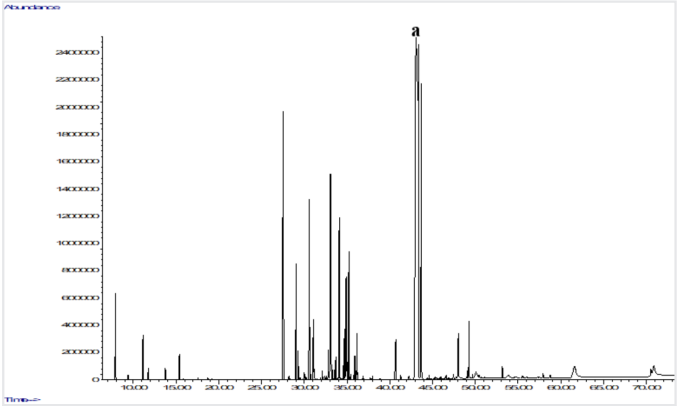

Figure 8. GC chromatogram of essential oil extracted from fruit of *D. carota* (D6) **a:** Carotol (74.6%)

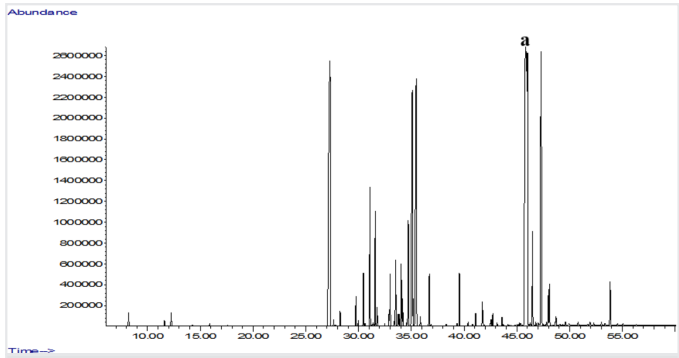

Figure 9. GC chromatogram of essential oil extracted from fruit of *D. carota* (D7) **a:** 11αH-himachal-4-en-1β-ol (49.4%)

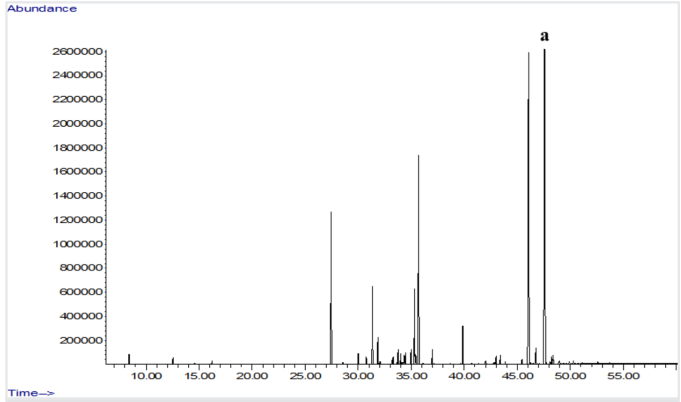

Figure 10. GC chromatogram of essential oil extracted from fruit of *D. carota* (D8) **a:** *Trans*-Methylisoeugenol (45.7%)

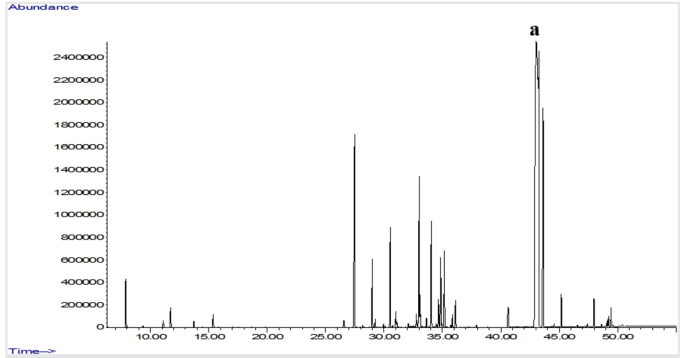

Figure 11. GC chromatogram of essential oil extracted from fruit of *D. carota* (D9) **a:** Carotol (63.1%)

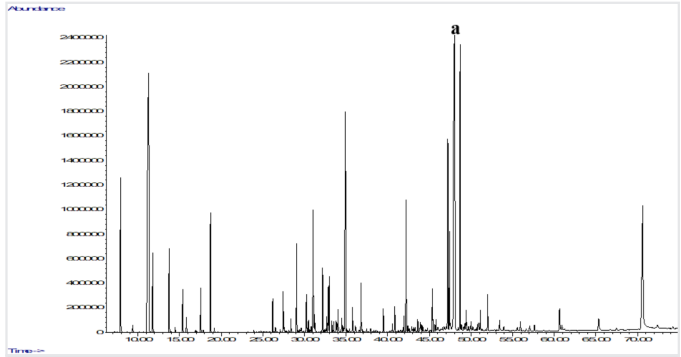

Figure 12. GC chromatogram of essential oil extracted from fruit of *D. carota* (D10) **a:** β-Sinensal (30.4%)

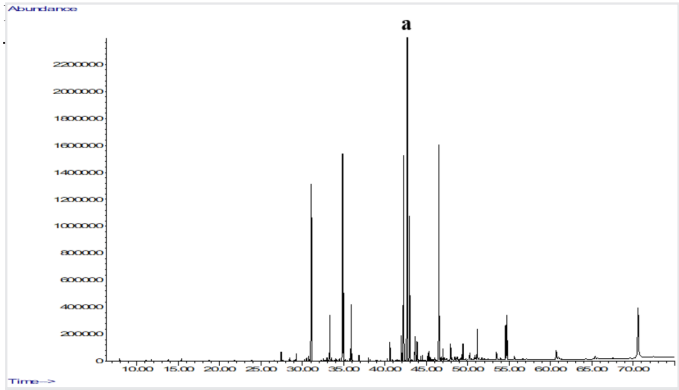

Figure 13. GC chromatogram of essential oil extracted from fruit of *D. carota* (D11) **a:** Methyl eugenol (30.5%)

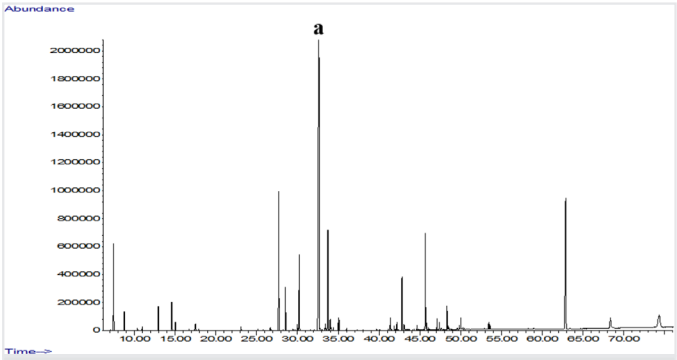

Figure 14. GC chromatogram of essential oil extracted from fruit of *D. carota* (D12) **a:** α-Humulene (29.4%)

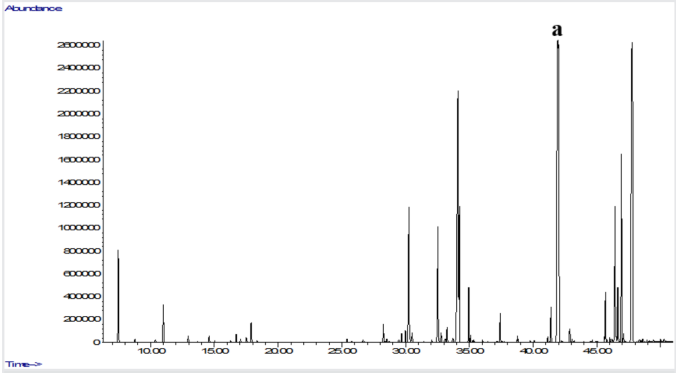

Figure 15. GC chromatogram of essential oil extracted from fruit of *D. carota* (D13) **a:** Methyl eugenol (40.9%)

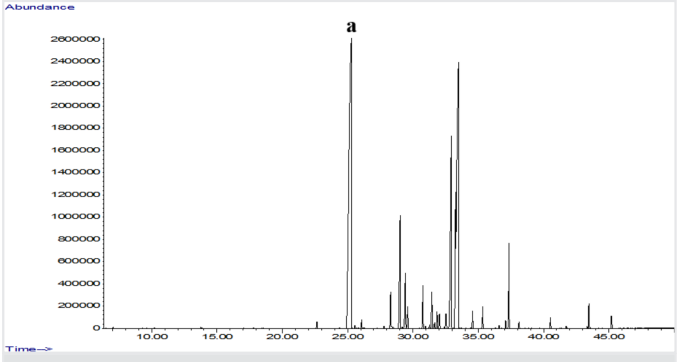

Figure 16. GC chromatogram of essential oil extracted from fruit of *D. carota* (D14) **a:** Methyl eugenol (29.6%)

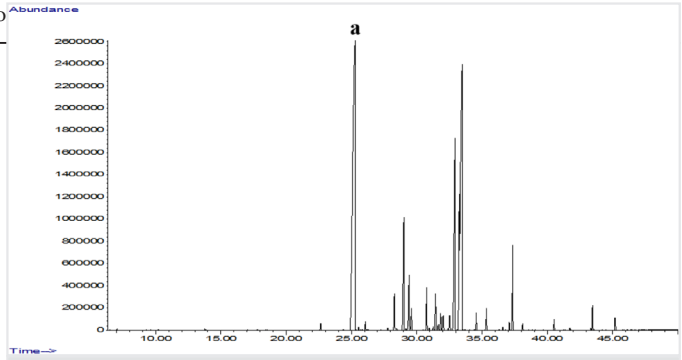

Figure 17. GC chromatogram of *D. carota* essential oil' *n*-hexane fraction (D7-H) **a:** Longipinene (33.5%)

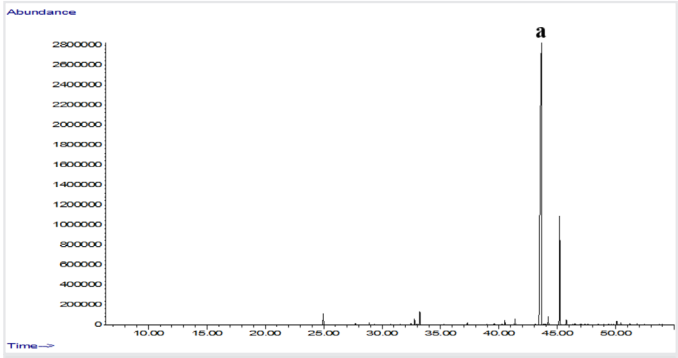

Figure 18. GC chromatogram of *D. carota* essential oil' ethanol (D7-E) **a:** 11αH-himachal-4-en-1β-ol (72.2%)

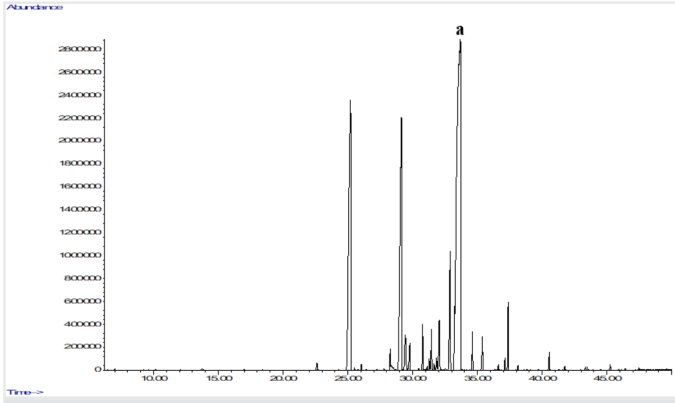

Figure 19. GC chromatogram of *D. carota* essential oil' *n*-hexane fraction (D8-H) **a:** β-Bisabolene (49.2%)

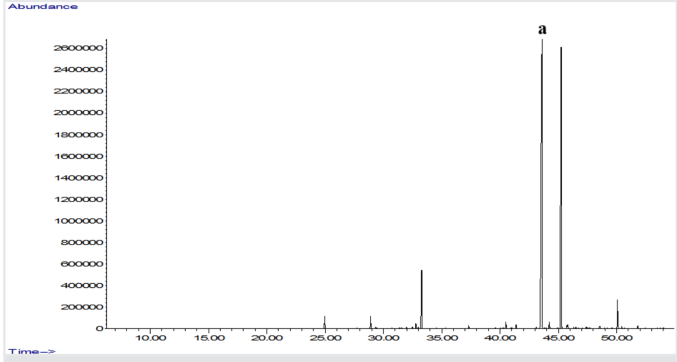

Figure 20. GC chromatogram of *D. carota* essential oil' ethanol fraction (D8-E) **a:** 11αH-himachal-4-en-1β-ol (52.0%)

Conclusion

In this study, the chemical composition of the fruit essential oils of *Daucus* species grown in Turkey was comparatively analyzed for the first time. The anti-inflammatory and anticholinesterase effects of the essential oils were investigated. Although there wasn't any anti-inflammatory activity in the samples, the anticholinesterase effect was observed in three samples of *D. carota* species' essential oils in our study. According to the results, it was seen that if the main components of the essential oil were standardized, it could be used in the preparation of potential pharmaceuticals and nutraceuticals. It is thought to be useful for complementary therapy, especially in neurodegenerative diseases.

Ethics

Ethics Committee Approval: Since there is no study related to teeth, ethics committee approval is not required.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: B.B.A., G.E.G., B.Z.K., B.D., Design: B.B.A., G.E.G., B.Z.K., B.D., Data Collection or Processing: B.B.A., G.E.G., B.Z.K., B.D., Analysis or Interpretation: B.B.A., G.E.G., B.Z.K., B.D., Literature Search: B.B.A., G.E.G., B.Z.K., B.D., Writing: B.B.A., G.E.G., B.Z.K., B.D.

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

Financial Disclosure: This study was supported by Anadolu University Scientific Research Projects Commission under the grant no: 1603S114 and Scientific Research Projects Coordination Unit of İstanbul University (project number: 22180).

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