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Chemical composition and biological activities of essential oils of *Foeniculum vulgare* Mill. and *Daucus carota* L. growing wild in Turkey

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ABSTRACT: The chemical composition of essential oils of aerial parts of *Daucus carota* (DEO) and *Foeniculum vulgare* (FEO) was specified by GC/MS. Antidiabetic, anti-inflammatory, antioxidant, and antibacterial activities of oils were tested by α-glucosidase inhibitory, 5-lipoxygenase inhibitory, DPPH/ABTS radical scavenging, and broth microdilution methods, respectively. Thirty components were identified in DEO (87.7%). Carotol (27.7%), elemicin (18.1%), and limonene (16.0%) were determined as the main compounds in DEO. Seventeen constituents were identified in FEO (100.0%). Estragole (34.0%), limonene (27.1%), and α-pinene (19.5%) were found as major components in FEO. Both oils displayed weak antibacterial activity against Gram-negative bacteria. However, the oils were not effective on Gram-positive bacteria. DEO with IC_{50} values of 1359 and 1579 µg/mL and FEO with IC_{50} values of 3106 and 1654 µg/mL showed weak antioxidant activities against DPPH and ABTS radicals, respectively. DEO and FEO exhibited good anti-lipoxygenase activities with IC_{50} values of 87.24 and 58.35 µg/mL, respectively. DEO presented significantly α-glucosidase inhibitory activity with an IC_{50} value of 44.78 µg/mL while FEO displayed poor activity with an IC_{50} value of 1967 µg/mL. This study is the first report on the 5-lipoxygenase and α-glucosidase inhibitory activities of aerial parts of DEO as well as on the α-glucosidase inhibitory activity of aerial parts of FEO. These results indicated that DEO, which has a good antidiabetic and anti-inflammatory activity, is rich in oxygenated sesquiterpene and monoterpene. Also, it showed that FEO, which has a significant anti-inflammatory activity, is rich in monoterpene and phenylpropanoid.

KEYWORDS: *Daucus carota*; *Foeniculum vulgare*; essential oils; biological activity.

1. INTRODUCTION

Foeniculum vulgare Mill. (Fennel) and *Daucus carota* L. (Wild carrot) are members of Apiaceae family. Fennel is a perennial herbaceous plant and grows 1-1.8 m. The flowers of the fennel are18-25 per umbellule and have yellow petals. The fruits of the fennel are ovoid-oblong-shaped, glabrous, and with prominent ridges. Fennel is spread mainly in West, South, and Central Europe, South-West Asia, and North Africa [1]. *F. vulgare* is called 'Rezene and Arapsaçı' in Anatolia used for the treatment of stomach discomfort, digestive facilitator, relieve gas, stomach and abdominal pains, against diarrhea and intestinal worms, colds, cough, chest softener, expectorant, kidney stone, urine enhancer, sedative, insomnia, eye itching, and milk secretion enhancer [2] due to its hepatoprotective effect, antioxidant, antithrombotic, anti-inflammatory, antidiabetic, antitumor, acaricidal, antifungal and antibacterial activities [3-4]. Fennel is a fragrance and delicious plant with culinary and therapeutic uses. The plant oils have a typical anise scent. The fennel oil had *trans*-anethole, fenchone, estragole (methyl chavicol), and α-phellandrene as the main components [3-5].

Wild carrot is a biennial plant and grows 10-200 cm. The flowers of wild carrot are white, pinkish, or yellowish petals [1]. The plant is a vegetable utilized in human alimentation. It is native to Europe, Asia, and Africa. *D. carota* is named 'Havuç, havuç tohumu, yere geçen, keşür and pörçüklü' [6] in Anatolia is used for the treatment of diabetes, eye diseases, skin disorders, constipation, against intestinal worms and

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hemorrhoids, getter, digestive facilitator, breast softener [2] due to antibacterial, antifungal, anthelmintic, hepatoprotective and cytotoxic activities [7]. The wild carrot oil is utilized as a diuretic and stomachic. The wild carrot oil has biological properties such as antimicrobial, hepatocellular regenerator, general tonic and stimulant, cholesterol regulatör, and cicatrizant [6]. The main compounds of oils of *D. carota* were carotol, sabinene, α-pinene, geraniol, β-bisabolene, γ-bisabolene, (*E*)-methyl isoeugenol, (*E*)-asorene, daucene and geranyl acetate [6,8]. There are many reports on the essential oil composition of *D. carota* and *F. vulgare* in the literature. However, there are no reports on the essential oil composition of *D. carota* growing wild in Turkey. Also, this study is the first report on the 5-lipoxygenase and α-glucosidase inhibitory activities of aerial parts of DEO as well as on the α-glucosidase inhibitory activty of aerial parts of FEO. This study aimed to specify the biological activities of the essential oils of *D. carota* and *F. vulgare*.

2. RESULTS

The yields of DEO and FEO were 0.88 and 0.77% (v/w), respectively. Thirty components were identified in DEO (87.7%). Carotol (27.7%), elemicin (18.1%), and limonene (16.0%) were determined as the main compounds in DEO (Figure 1 and Table 1). The dominant groups of DEO were oxygenated sesquiterpenes (32.4%), monoterpene (26.9%), and phenylpropanoid (18.5%) (Table 1). Seventeen constituents were identified in FEO (100.0%). Estragole (34.0%), limonene (27.1%), and α-pinene (19.5%) were found as major components in FEO (Figure 2 and Table 1). FEO had monoterpene (56.6%) and phenylpropanoid (34.0%) as major groups (Table 1). DEO and FEO displayed weak antibacterial activity against Gram-negative bacteria. However, the oils were not effective on Gram-positive bacteria (Table 2).

DEO and FEO showed poor DPPH radical scavenging activity with IC_{50} values of 1359 and 3106 µg/mL compared to the IC_{50} value of standard ascorbic acid, 17.60 µg/mL. Also, DEO and FEO showed low ABTS radical scavenging activity with IC_{50} values of 1579 and 1654 µg/mL compared to the IC_{50} value of standard ascorbic acid, 13.00 µg/mL (Table 3). DEO and FEO with IC_{50} values of 87.24 and 58.35 µg/mL displayed good anti-lipoxygenase activity compared to standard indomethacin with an IC_{50} value of 22.39 µg/mL, respectively (Table 3). DEO and FEO presented significant and weak α-glucosidase inhibitory activity with IC_{50} values of 44.78 and 1967.00 µg/mL compared with that of acarbose (40.06 µg/mL), respectively (Table 3).

3. DISCUSSION

The essential oil of *F. vulgare* possessed remarkable diversities in the main compounds. These diversities could be related to morphological characteristics and different geographical origins. Estragole, fenchone, and limonene were determined as main compounds in the volatile oil of *F. vulgare* growing wild in Turkey [9-11]. Fenchone is utilized as a counter-irritant and found a low amount in the oil of the current report, α-pinene is used as insecticides, solvents, and perfume bases [12]. This compound was determined in a low amount (1.0-1.5%) in the previous reports [9-11]. The essential oil of cultivated *F. vulgare* of Turkish origin possessed *trans*-anethole as the main compound [13-18]. However, this compound was not determined in the oil of the present study. The absence of *trans*-anethole in the present study together with the low content of α-pinene in previous studies suggests these differences are observed due to different chemovarieties of the plant. In the present report, the volatile oil of aerial parts of *F. vulgare* showed a similar chemical profile with *F. vulgare* growing wild in Turkey but there are quantitative differences in the major components of essential oils.

The chemical composition of essential oils of *D. carota* showed variations during ontogenesis. Especially, the phenylpropanoid contents are found in high amounts in the mature umbels [19]. Carotol was determined as the main compound in the essential oil of cultivated *D. carota* from Turkey. Also, limonene was found in a low amount (0.4%) and elemicin was not detected in the oil of the previous study [20]. The present study showed that have similar essential oil components to previous studies. However, there are quantitative dissimilarities in the major components of the oils.

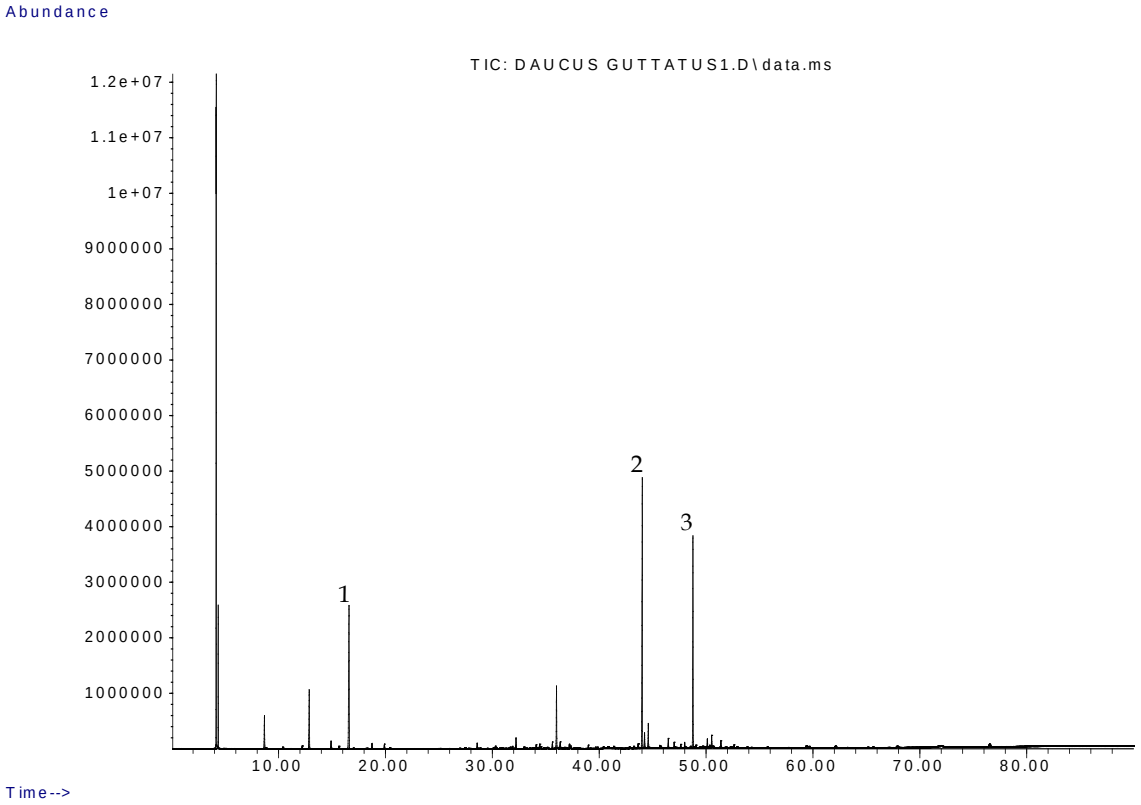

Figure 1. GC-MS chromatogram of DEO (1: Limonene; 2: Carotol; 3: Elemicine).

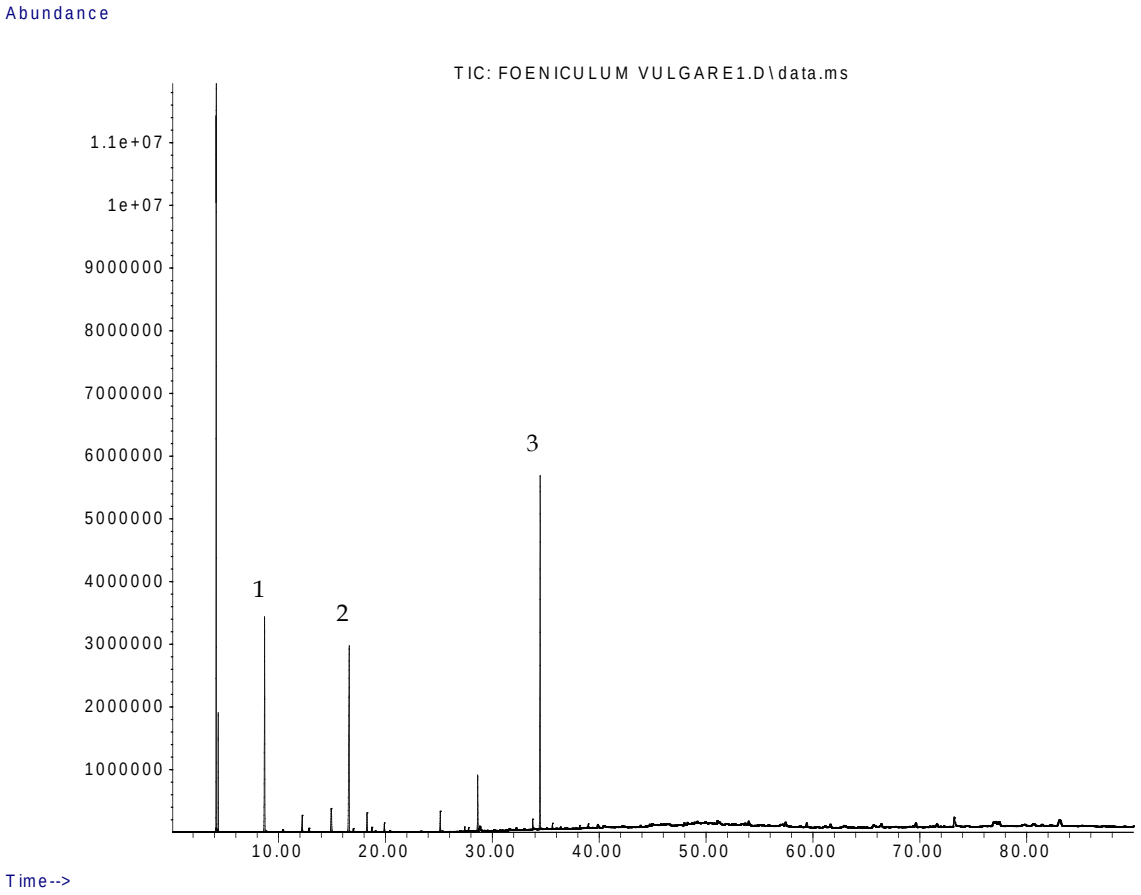

Figure 2. GC-MS chromatogram of FEO (1: α-pinene; 2: Limonene; 3: Estragole).

Table 1. The essential oil composition of aerial parts of *Daucus carota* and *Foeniculum vulgare*.

RRI[1]	RRI Lit.[2]	Compounds	*D. carota* (%)	*F. vulgare* (%)
1020	**1032**	**α-Pinene**	**2.8**	**19.5**
1064	1076	Camphene	0.2	-
1109	1118	β-pinene	0.3	1.9
1123	1132	Sabinene	5.8	0.4
1165	1174	Myrcene	0.7	-
1166	1176	α-Phellandrene	-	3.8
1181	1188	α-Terpinene	0.2	-
1200	**1203**	**Limonene**	**16.0**	**27.1**
1209	1218	β-Phellandrene	-	0.4
1237	1246	β-(Z)-Ocimene	-	2.0
1248	1255	γ-Terpinene	0.5	0.5
1274	1278	*p*-Cymene	0.4	1.0
1400	1406	Fenchone	-	2.0
1463	1468	*Trans*-limonene oxide	-	0.4
1474	1482	α-Fencyl acetate	-	0.4
1548	1553	β-linalool	0.2	-
1590	1594	*Trans*-α-bergamotene	0.1	-
1596	1600	β-Elemene	0.2	-
1597	1591	Fencyl alcohol	-	5.1
1607	1607	Terpinen-4-ol	1.0	-
1632	1639	*Trans*-*p*-mentha-2,8-dienol	0.1	-
1669	1668	(Z)-β-Farnesene	0.3	-
1680	**1687**	**Estragole**	**0.4**	**34.0**
1684	1683	*Trans*-verbenol	0.2	-
1720	1726	Germacrene D	0.6	0.5
1734	1737	β-Bisabolene	5.4	-
1746	1751	Carvone	0.9	-
1780	1784	α-Bisabolene	0.2	-
1840	1845	*Trans*-carveol	0.3	0.4
2020	2050	(E)-Nerolidol	0.7	-
2036	**2045**	**Carotol**	**27.7**	**-**
2137	2144	Spathulenol	0.9	-
2237	**2245**	**Elemicine**	**18.1**	**-**
2297	2284	Daucol	0.9	-
2314	2313	Juniper camphor	1.3	-
2350	2369	(2E,6E)-Farnesol	0.9	-
2613	2622	Phytol	0.4	0.6
		Monoterpenes	26.9	56.6
		Oxygenated Monoterpenes	2.7	8.3
		Sesquiterpenes	6.8	0.5
		Oxygenated Sesquiterpenes	32.4	-
		Diterpenes	0.4	0.6
		Phenylpropanoid	18.5	34.0
		Total identified compounds	**87.7**	**100.0**

[1]RRI: Relative retention time;
[2]RRI Lit: Relative retention index of the compound given in the literature.

Table 2. Antibacterial activity of essential oils of *Daucus carota* and *Foeniculum vulgare*.

Essential oils	Gram-positive bacteria		Gram-negative bacteria	
	Staphylococcus aureus	*Bacillus subtilis*	*Klebsiella pneumoniae*	*Escherichia coli*
D. carota	>18 mg/mL	>18 mg/mL	18 mg/mL	18 mg/mL
F. vulgare	>18 mg/mL	> 18 mg/mL	9 mg/mL	18 mg/mL

Table 3. Antioxidant, anti-inflammatory, and antidiabetic activities of DEO and FEO.

Essential oils and standards	Antioxidant activity		Anti-inflammatory activity	Antidiabetic activity
	DPPH radical scavenging activity	ABTS radical scavenging activity	Anti-lipoxygenase activity	α-glucosidase inhibitory activity
	IC_{50} (µg/mL)			
DEO*	1359 ±7.07[b]	1579 ± 1.41[b]	87.24 ± 4.34[c]	44.78 ± 0.31[a]
FEO*	3106 ± 9.19[c]	1654 ± 2.83[c]	58.35 ± 0.92[b]	1967.00 ± 7.07[b]
Ascorbic acid	17.60 ± 0.37[a]			
Trolox		13.00 ± 0.21[a]		
Indomethacin			22.39 ± 0.26[a]	
Acarbose				40.06 ± 2.14[a]

*Abbreviations: DEO and FEO show essential oils obtained from aerial parts of *Daucus carota* and *Foeniculum vulgare,* respectively. **Each value in the table is represented as mean ± SD (n=3). Different letter superscripts in the same column indicate significantlydifferences (p<0.05).

(*E*)-Methyl isoeugenol, α-pinene, elemicin, carotol, sabinene, limonene, β-pinene, myrcene, 11α-H-himachal-4-en-1β-ol, β-bisabolene, geranyl acetate, *trans*-anethole, (*E*)-β-ocimene, caryophyllene, *epi*-bicyclosesquiphaellandrene, terpinen-4-ol, shyobunone, β-cubebene, preisocalamendiol, isoelemicin, and myristicin were determined as main compounds in the oils of *D. carota* of other geographical origins [19-47]. The similarities and dissimilarities were observed between essential oil constituents of the present and previous studies. (*E*)-methyl isoeugenol, 11α-H-himachal-4-en-1β-ol, geranyl acetate, *trans*-anethole, (*E*)-β-ocimene, caryophyllene, *epi*-bicyclosesquiphaellandrene, shyobunone, β-cubebene, preisocalamendiol, isoelemicin, and myristicin were not determined in the oil of the present study. The previous researches specified that *D. carota* had monoterpene, oxygenated monoterpene, sesquiterpene, oxygenated sesquiterpene, and phenylpropanoid as dominant groups. In the present report, the oil of aerial parts of *D. carota* showed that have similar dominant groups with the previous researches.

The variations of essential oil ingredients and composition may be connected to factors such as plant parts used, geographical regions, genotype, ecotype, chemotype, phenophases, and the environment. The environmental factors can be temperature, relative humidity, irradiance, and photoperiod. The quantitative composition of the volatile oils of numerous aromatic plants is significantly influenced by the harvesting time, plant age, and product density [48].

In studies conducted by Staniszewska et al. (2005) and Jabrane et al. (2009) determined that *D. carota* oil was effective on Gram-positive and Gram-negative bacteria [42,46]. In another study, Glišić et al. (2007) and Asilbekova et al. (2017) reported that *D. carota* essential oil showed strong activity against Gram-positive bacteria but was not effective on Gram-negative bacteria [43,47]. The results of the current study did not support the literature. The previous studies had monoterpene and oxygenated sesquiterpene as dominant groups. They did not include the phenylpropanoid group as a high amount as with the present study. The results of the current study might be due to the synergistic activity of the phenylpropanoid group and other major groups present in the oil. Diao et al. (2014), Gulfraz et al. (2008), Anwar et al. (2009), Roby et al. (2012), Dadalioglu and Evrendilek (2004) reported that *F. vulgare* essential oil was effective on Gram-positive and Gram-negative bacteria at different MIC values [3,49-52]. The results of the current study did not support the literature. *Trans*-anethole was the main compound in the oil of previous studies. This compound was not detected in the oil of the current study. The antimicrobial activity of six essential oils of *F. vulgare* fruits from different locations of Portuguese was studied. Fenchone, estragole, and *trans*-anethole were determined as the main compounds in different percentages. Limonene and α-pinene were found in a low amount in estragole chemotype oils. And estragole chemotype oils were effective on Gram-positive and Gram-negative bacteria at different MIC values [53]. The antibacterial activity results of the current study showed a dissimilar profile from the previous study. This dissimilar profile could be related to the different percentages of the main compounds in the oils.

In a study conducted by Meliani et al. (2012), it was found that hydro-distilled essential oil obtained from the aerial parts of *Daucus carota* subsp. *carota* had weak antioxidant activity against DPPH radical with IC_{50} value of 40.97 mg/mL [28]. In another study, Mohammedi et al. (2015) reported that essential oils of *D. carota* subsp. *carota* aerial parts before flowering and after flowering had a low antioxidant activity with IC_{50} values >200 µg/mL. In the same study, it was found that the essential oil of *D. carota* subsp. *carota* aerial parts

in full flowering had a good activity with an IC_{50} value of 96.4 µg/mL against the DPPH radical [54]. Except for the last report, the results of previous studies are in line with the results of our current study. Felice et al. showed that essential oil of *F. vulgare* Mill. subsp. *vulgare* var. *azoricum* leaves had a weak antioxidant capacity against DPPH free radical [55]. In another study, it was found that all essential oils from dried aerial parts *F. vulgare* with different hydrodistillation times had low DPPH radical scavenging activity with an inhibition rate below 40 % at the concentration of 1000 µg/mL [56]. Also, Albano et al. reported that the essential oil of *Foeniculum vulgare* aerial part had an IC_{50} value of 2342.0 µg/mL against DPPH radical [57]. These studies support the results of our current study.

Albano et al. indicated that essential oil of *F. vulgare* aerial part had a good anti-5-lipoxygenase activity with an IC_{50} value of 67.7 µg/mL [57]. Although there are no reports in the literature regarding the anti-inflammatory activity of the essential oil obtained from the aerial part of *D. carota* by lipoxygenase inhibition method, there are studies with different methods. In one of these studies, Silva evaluated the anti-inflammatory activity of the essential oil on NO release in LPS-stimulated macrophages and reported that essential oil was able to reduce by 19% the NO production in LPS-stimulated macrophages at 0.64 µL/mL without showing toxicity to the cells [58]. These studies also showed that both oils had significant anti-inflammatory activity and were consistent with the results of our current study. Also, Frum and Viljoen revealed limonene had 5-lipoxygenase inhibitory activity [59]. Previous studies have suggested that α-pinene [60], elemicin [61], estragole [62] had anti-inflammatory effects. Therefore, these major compounds found in DEO and FEO and other compounds may be responsible for the anti-inflammatory activities of the oils.

There is no antidiabetic activity study on DEO in the literature. However, it was reported that the ethanol extract obtained from the seeds of *D. carota* had antidiabetic activity [63]. Similar to DEO, there are no reports on essential oil obtained from aerial parts of *F. vulgare* but there are studies in the literature on the essential oil obtained from its seed. In one of these studies, El-Soude et al. evaluated the essential oil of seed of *F. vulgare* for its hypoglycaemic effect and found that the oil had good antidiabetic activity [64]. Also, previous studies have shown that limonene has antidiabetic activity [65]. Therefore, limonene and other compounds may be responsible for the activity of the essential oil obtained from DEO, which exhibits an important antidiabetic activity in our current study.

4. CONCLUSION

These results showed that FEO against 5-lipoxygenase and DEO against α-glucosidase and 5-lipoxygenase had good inhibitory activity. However, *in vivo* studies are needed to definitively determine the therapeutic efficacy of oils.

5. MATERIALS AND METHODS

5.1. Plant material

Daucus carota and *F. vulgare* were collected in İkitelli-Başakşehir (41° 04' 34.9'' N; 28° 47' 32.2'' E), Istanbul, Turkey, in June 2017 by Huseyin Servi Ph.D and identified by Dr. Ahmet Dogan. Herbarium specimens of *D. carota* and *F. vulgare* were deposited in the Marmara University Herbarium (Voucher specimens numbers: MARE 22156 and MARE 20233).

5.2. Essential oil analyses

The aerial parts of DEO (100 g) and FEO (114 g) were subjected to hydrodistillation for 3 h, using a Clevenger apparatus. DEO and FEO were stored in amber vials under -20°C until analyzed.

5.3. GC/MS analysis

The GC/MS analysis and determination of essential oils components were performed as described by Servi et al. [66]. In GC/MS analyses, the Innowax FSC column (60 m×0.25 mm, 0.25 m film thickness) and helium as carrier gas (1 mL/min) were used. The identification of the essential oil components was carried out by comparison of their relative retention indices obtained by series of *n*-alkanes (C5 to C30) to the literature and with mass spectra comparison. The mass spectra comparison was done by computer matching with the

commercial Wiley 8th Ed./NIST 05 Mass Spectra library, Adams Essential Oil Mass Spectral Library, and Pallisade 600K Complete Mass Spectra Library.

5.4. Gas Chromatography

The GC analyses were done with an Agilent 6890N GC system. FID detector temperature was set to 300°C and the same operational conditions were applied to a duplicate of the same column used in the GC-MS analyses. Simultaneous autoinjection was used to obtain the same retention times. The relative percentage amounts of the separated compounds were calculated from integration of the peaks in the FID chromatograms.

5.5. Biological activity

5.5.1. Antibacterial activity

The antibacterial activity of the essential oils was evaluated against *Klebsiella pneumoniae* ATCC BAA-1706, *Escherichia coli* ATCC 14169, *Staphylococcus aureus* ATCC 25923, and *Bacillus subtilis* ATCC 19659 by using a broth microdilution [67]. The stock solution of the oils (18 mg/mL) was prepared with %10 dimethyl sulfoxide (DMSO). Serial dilution of essential oil was done on 96-well microplates using Mueller Hinton Broth (MHB). Bacteria were standardized in MHB according to McFarland No:0.5. Bacterial cultures were mixed with different concentrations of essential oils on microplates and were incubated 24 h at 37°C. Minimum inhibitory concentrations (MIC: mg/mL) were detected at the minimum concentration where bacterial growth was not detected. Chloramphenicol was used as a positive control for assays. All the experiments were performed in duplicate.

5.5.2. Measurement of DPPH radical scavenging activity

The free radical scavenging activity of the essential oils, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by Zou et al. [68]. Briefly, 10 µL of essential oils (250-0.49 µg/mL) or standard ascorbic acid (100-0.02 µg/mL) in DMSO at different concentrations were mixed with 190 µL of 0.1 mM DPPH solution in MeOH in a well of 96-well plate. The reaction mixture was left in the dark at RT for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Each experiment was carried out in triplicate.

5.5.3. Measurement of ABTS radical-scavenging activity

The ABTS radical cation-scavenging activity was performed according to the method described previously [68]. ABTS radical cations were generated by mixing an equal volume of ABTS (7 mM in H_2O) and potassium persulfate (4.9 mM in H_2O), allowing them to react for 12-16 h at room temperature in the dark. ABTS radical solution was diluted with 96% ethanol to an absorbance of about 0.7 at 734 nm. 10 µL of essential oils (250-0.49 µg/mL) or standard Trolox (100-0.02 µg/mL) were added to 190 µL of ABTS radical solution in a 96-well microplate. The reaction mixture was kept in the dark for 30 min. Then, the absorbance was recorded at 734 nm. All measurements were carried out in triplicate.

5.5.4. Measurement of in vitro anti-inflammatory activity

5-lipoxygenase inhibition activity was analyzed by the method of Phosrithong and Nuchtavorn [69] with slight modifications described by Yıldırım et al. [70]. 10 µL of essential oils (250-0.49 µg/mL) or standard indomethacin (100-0.02 µg/mL) were added to 20 µL ethanol, 20 µL pure water, 25 µL of sodium borate buffer solution (0.1 M, pH 9) and 25 µL of type V soybean lipoxygenase solution in the buffer (pH 9, 20.000 U/mL). The reaction mixture was pre-incubated at 25 °C for 5 min. Then, 100 µL of 0.6 mM linoleic acid solution was added to solutions, mixed well and the change in absorbance at 234 nm was followed for 6 min. Each reaction was run in triplicate.

5.5.5. Measurement of in vitro antidiabetic activity

The inhibition assay for α-glucosidase activity was conducted as described by Ramakrishna et al. [71] with slight modifications described by Sen et al. [72]. In a 96-well plate, 10 µL of essential oils (250-0.49 µg/mL) or standard acarbose (100-0.02 µg/mL), 40 µL of 0.1 M sodium phosphate buffer (pH 6.9), and 100 µL of α-glucosidase (obtained from *Saccharomyces cerevisiae*) were mixed. After pre-incubation at 25°C for 10 min, 50 µL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside (pNPG) to the solutions was added and re-incubated at 25 °C

for 5 min. The absorbance reading was taken before and after incubation at 405 nm using a microplate reader. Tests were carried out in triplicate.

5.6. Statistical analysis

The data were reported as means±standard deviations and analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests using GraphPad Prism 5. Differences between means at $p<0.05$ level were considered significant.

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